SOLVAY AMERICA

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ORIGINAL

United States Environmental
Protection Agency
Document Processing Center (7407)
Office of Pollution Prevention and Toxics
Room 105, East Tower
401 M Street, S.W.
Washington, D.C. 20460

89950000171

Mail
Receipt Requested

Attention: TSCA Section 8(e) Coordinator

Re: DuPont Submission No. 8EHQ-0295-13318

Contains No CBI

Dear Sir:

This letter and the enclosures are being submitted in response to the letter from Mr. Oscar Hernandez, Chief, Risk Analysis Branch, dated February 23, 1995. To expedite a response and as a courtesy, I am responding directly to Mr. Hernandez's request. Solvay America, Inc. is not involved in the manufacture, sale, distribution or use of 1,1,2-trifluoroethane (HFC-143), or in the project which led to the development of the data sought.

As you are aware, the summaries of three toxicity reports involving 1,1,2-trifluoroethane (HFC-143) (the "Reports") were submitted to your office by DuPont Central Research and Development ("DuPont") pursuant to Section 8(e) of the Toxic Substances Control Act, as amended ("TSCA"). EPA assigned the above-referenced submission number to the DuPont filing.

As noted in the DuPont filing, the Reports were sponsored by Solvay Duphar B.V. ("Duphar"), one of our European affiliates. Duphar furnished the summaries of the Reports directly to DuPont. Mr. Hernandez wrote to me requesting copies of the final Reports which request was forwarded on to Duphar to expedite maters. Having just received the Reports from Duphar, I am pleased to satisfy his request. The following documents are enclosed:

1. Solvay Duphar B.V. (February, 1994) *14-Day Inhalation Study of 1,1,2-Trifluoroethane (HFC 143) in Male and Female Rats*, Report No. S 9314 (plus Addendum).

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6/12/96

Solvay America, Inc.
3333 Richmond Avenue, Houston, Texas 77098

2. Solvay Duphar B.V. (May, 1994) *Study of the Testicular Toxicity in Male Rats after a Single Inhalatory Exposure to 1,1,2-Trifluoroethane (HFC-143)*, Report No. S.9316.

3. Solvay Duphar B.V. (December 21, 1994) *HFC 143 (Trifluoroethane) 28-Day Inhalation Toxicity Study in Rats*, Report No. S.9410.

Questions regarding the Reports or their contents can only be answered by Duphar. If you should require additional assistance, we would be pleased to forward any requests to Duphar. If you should have any questions, please do not hesitate to contact the undersigned at (713) 525-6026.

Very truly yours,

Paul J. Harding
Attorney

PJH/jlv

Enclosures

cc: Dr. Charles F. Reinhardt
DuPont Central Research and Development
(w/encls.)

SOLVAY DUPHAR B.V.

Weesp, The Netherlands
Department of Toxicology
Int.Doc.No. 56345/43/93
Report No. S.9314
Issued February 1994

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUORO-ETHANE (HFC 143) IN MALE AND FEMALE RATS

Authors: H.J.S. Koelman
P.J.M. Janssen
R.L.F. Dawes
M. de Haan

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Total number of pages is (vii) + 100

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LIST OF ABBREVIATIONS

Album	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
Bil	bilirubin
Chol	cholesterol
Creat	creatinin
EDTA	ethylene diaminetetra-acetic acid
Ery	blood
GGT	γ-glutamyltranspeptidase
GLP	Good Laboratory Practice
Glu(c)	glucose
Hb	haemoglobin
HCT	packed cell volume
HGB	haemoglobin
In.pho	inorganic phosphate
Ket	ketones
LEU	leucocytes
Lymph	lymphocytes
MCH	mean cell haemoglobin
MCHC	mean cell haemoglobin concentration
MCV	mean cell volume
n	number
NAG	N-acetyl-b-D-glucosaminidase
Nit	nitrate
NOEL	no-effect-level
Osmo	osmolality
PCV	packed cell volume
Plt	platelets
Pro(t)	protein
QA	Quality Assurance
RBC	red blood cells
RH	relative humidity
Sd (sd)	standard deviation
t	temperature
Trig	triglycerides
Ubg	urobilinogen
w/v	weight/volume
WBC	white blood cells

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STATEMENT OF GLP COMPLIANCE

With respect to the following study:

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS

I, the undersigned, hereby declare that this report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The study, performed in the Department of Toxicology of SOLVAY DUPHAR B.V., Weesp, The Netherlands, was conducted in accordance with:

- Good Laboratory Practice in the Testing of Chemicals, Good Laboratory Practice Principles, Organization for Economic Cooperation and Development (OECD), 1982, including all supplements published up to the starting date of this experiment.

Study director:

H.J.S. Koelman date: 14 February 1994

Head of the Department of Toxicology:

F.M.H. Debets date: 14 Febr '94

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QA-STATEMENT

The following report has been audited by the Quality Assurance Unit of Solvay Duphar B.V.

Report No.	:	S.9314
Int.Doc.No.	:	56345/43/93
Title of the report	:	**14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS**
Author	:	E. Koelman

The audit included the comparison of the individual data reported with the data recorded in notebooks, work sheets and other relevant papers.

This report has been accepted by the Quality Assurance Unit as being an accurate presentation of the individual findings of the study.

Date of inspection/audit	Date of report to management
29 APR 93	29 APR 93
25 MAY 93	25 MAY 93
10 JUN 93	11 JUN 93
17 JUN 93	17 JUN 93
23 JUN 93	23 JUN 93
29 SEP 93	05 OCT 93
23 DEC 93 EN 24 DEC 93	24 DEC 93
16 FEB 94	16 FEB 94

C.J.M. van Gasteren
Head of the Quality Assurance Unit

Weesp, 22 FEB 94

Weesp, The Netherlands
Department of Toxicology
Int.Doc.No. 56345/43/93
Report No. S.9314
Issued February 1994

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS

1. SUMMARY

The aim of this study was to investigate the toxicological effects, which can be observed in rats during and after a 14-day nose-only inhalatory exposure to 1,1,2-trifluoroethane (HFC 143) at a target exposure level of 10,000 ppm and to get an indication of the range of exposure levels for future studies. Two groups of ten male and ten female rats were exposed for 14 days, 5 days/week, 6 hours/day to a test atmosphere containing 0 and 10,000 ppm HFC 143. The following parameters were included: clinical signs, body weight, food and water consumption, haematology, clinical chemistry, urine analysis, organ weights, macroscopy and histopathology.

In the present study the main effects were: three compound related mortalities among the females; (generally slight) clinical signs (indicative of central nervous system and locomotor disturbances) and reduced weight-gain and food consumption in both sexes; increased water consumption in the females; decreased lymphocyte counts in the males; increased number of normoblasts in both sexes; minor effects on alkaline phosphatase (males decreased, females increased); increased plasma level of inorganic phosphate in the females; reduced urinary protein excretion in the males; increased urine volume in the females; increased urinary fluoride concentration and total excretion in both sexes; severely reduced testis weights; increased adrenal and lung weights in the females; macroscopic changes in the lungs of decedent females; microscopic liver changes in the decedent females, and lung changes in the males; severe testicular atrophy with degenerated spermatids and giant cells.
No effects were found on the plasma oestradiol and testerone levels.

From the results of this study the following conclusions were drawn:

- HFC 143, when given to Sprague Dawley rats by the inhalatory route at 10,000 ppm for 2 weeks, 5 days per week, 6 hours per day, resulted in clear toxicity. Three females died within 6 days with clinical signs comparable to other treated females. The major target organ appeared to be the testis. Other target organs identified are the lungs and possibly the adrenals. Most relevant other parameters affected were clinical signs; body weight and food consumption (reduced); increased water consumption, associated with increased urine volume (in females); lymphocytes (decreased in males); and effects on the erythropoietic system.

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- For future repeated dose studies, dose levels higher than 10,000 ppm are not recommended. In view of the severe testis effects, no recommendation for low dose levels can be given, as no estimation of a NOEL can be made.

- It is recommended to investigate whether the testis effects also occur after single exposure, whether these effects are reversible (after single exposure and, if so, also after repeated exposure) and to assess the NOEL for this effect.

- Urinary fluoride concentration might well be a useful parameter for bio-monitoring purposes.

Head of the Department of Toxicology:

F.M.H. Debets 14 Febr '94 date

Authors:

H.J.S. Koelman 14 February 1994 date

P.J.M. Janssen 14 February 199 date

R.L.F. Dawes 14 February 1994 date

M. de Haan 14 February 199 date

2. INTRODUCTION

2.1 Study objective

The aim of this study was to investigate the toxicological effects, which can be observed in rats during and after a 14-day inhalatory study (10 exposure days) to 1,1,2-trifluoroethane (HFC 143) at an exposure level of 10,000 ppm and to get an indication of the range of exposure levels for future studies. HFC 143 is regarded as an alternative for fully halogenated chlorofluorocarbons. In previous studies with comparable chemicals the central nervous system, kidneys, adrenals and testes were identified as possible target organs.

The study was carried out on request of Solvay S.A., Rue de Ransbeek 310, 1120 Brussels, Belgium.

2.2 Dates and place of performance of the study

The study (DT 93/21) was carried out in the laboratory of Solvay Duphar B.V., Department of Toxicology, C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands. The in-life part of the study started on 25 May 1993, when the animals arrived and ended on 17th June 1993, the day of terminal necropsy.

2.3 Personnel involved

The study was carried out under the supervision of H.J.S. Koelman (study director) and P.J.M. Janssen (deputy study director). Necropsy was supervised by R.L.F. Dawes (pathologist); haematology, clinical chemistry and urine analysis by M. de Haan (clinical chemist). The in-life part of the study was supervised by W.M. van Doorn (inhalation technician) and T.E. Busé-Pot (animal technician).

2.4 Good Laboratory Practices

The study was conducted in accordance with:
Good Laboratory Practice in the Testing of Chemicals, Good Laboratory Practice Principles, Organization for Economic Cooperation and Development (OECD), 1982, including all supplements published up to the starting date of this experiment.

2.5 Archives

The raw data, the specimens and the master copy of the final report are stored in the archives of Solvay Duphar B.V., C.J. van Houtenlaan 36, 1381 CP, The Netherlands

3. MATERIALS AND METHODS

3.1 Test material

HFC 143, a gas at room temperature, was supplied by the study sponsor in a steel gas bottle. Prior to dosing the test material was stored at room temperature in a fume cupboard or a well ventilated room.

Chemical name	: 1,1,2-trifluoroethane
Structural formula	: CH_2F-CHF_2
Synonyms	: HFC 143
CAS-number	: 430-66-0
Molecular weight	: 84.04
Appearance	: Gas at room temperature (approx. 22°C)
Lot no.	: 71914/5
Purity	: > 99%
Boiling point	: 5°C

3.2 Test animals

Twenty-nine male and twenty-eight female Sprague Dawley rats (age at arrival: 63 to 70 days) were obtained from Charles River Wiga GmbH, Sulzfeld, Germany. The animals were inspected for signs of illness at the beginning of the acclimatization period. No abnormalities were detected. Twenty male and twenty female rats were used in the study. Seven animals per sex were used for validation of testosterone and oestradiol radioimmunoassays. Remaining animals were transferred to the laboratory animal pool for use in other studies.

3.3 Animal care

At their arrival the animals were housed in animal room 309, CDA III. The animals were housed individually in stainless steel wire mesh cages under the following conditions:

Temperature	: mainly 20-22°C (min. 19°C; max. 23°C)
Relative humidity	: mainly 50-70% (min. 54%; max. 87%)
Day/night cyclus	: 12 hours light / 12 hours dark.
Radiosound	: during light period.
Ventilation	: approximately 16 air changes per hour.

In these cages the animals had free access to powdered rodent diet (Biosure LAD-2, SDS, Witham, England) and water. The latter was available from macrolon drinking bottles. No food and water were supplied during the periods from removal from the cages until replacing back into the cages in view of the 6-hour exposure periods. No food was supplied during urine collection. Animal room and equipment were cleaned according to standard operating procedures.

The certificate of analysis of the batch of diet used for the study is presented in appendix 4. Drinking water is analyzed periodically. A representative certificate of analysis is presented in appendix 5. There were no known contaminants in food or water that were considered likely to interfere with the study.
In addition, to assist in the evaluation of the fluoride excretion, one sample was taken from one randomly selected water bottle per group per sex; these samples were analyzed for fluoride content only.

The animals were acclimatized for nine days prior to the start of exposure.

3.4 Treatment groups and allocation procedure

Two days after arrival, the animals were weighed. After exclusion of the lightest and heaviest animals, per sex ten animals were allocated to the control group and ten to a test group, using a stratified randomization according to body weight. The aim of this procedure was to minimize the variation in body weight at the start of exposure. For randomization of the groups over the cages, a randomized block design, was used. To all cages colour-coded tags (control white, test group red) were attached indicating cage, group and animal number. After randomization, the animals were identified by tail-tattoo, according to their cage numbers (1 through 20, per sex). In addition all animals in the HFC 143-treated group were identified with an area of picric acid on their backs.

From the remaining animals seven males and seven females were randomly selected for validation purposes (see 3.2). The remainder of the animals were transferred to the laboratory animal pool for use in other studies.

3.5 Treatment

The animals were exposed to the test atmosphere (see 3.6.1) for 6 hours/day, 5 days/week for 2 weeks (monday through friday). The target concentration of the treatment group was 10,000 ppm.
For exposure the animals were placed in plastic restraining tubes (Batelle, Geneva, Switzerland) which were fitted with the front end to the exposure chamber so that only the snout of the animal came in contact with the test atmosphere.

3.6 Exposure

3.6.1 Exposure chamber

The control animals were exposed to pressurized air (flow approx. 10 l/min) in a cylindrical nose-only exposure chamber (height 12 cm, diameter 38 cm, volume 13.6 l). The ports for the restraining tubes are located in the side wall of the exposure chamber.

The treated animals were exposed to the test atmosphere (flow approx. 10 l/min) using an aluminium cylindrical nose-only exposure chamber (height 55 cm, diameter 33 cm, volume 47 l). This chamber is constructed from aluminium and the inside wall is coated with silver and a thin layer of polytetrafluoroethylene. The chamber consists of three sections of approximately equal dimensions. Ports for the restraining tubes are located in the side wall of the middle section. The test atmosphere was generated by mixing a flow of test material with a stream of pressurized air in a Y-shaped manifold (Festo).

In both chambers the inlet and outlet are located in the centre of the bottom and top of the exposure chamber respectively. In addition, in the tubing at the outlet a probe for monitoring temperature and relative humidity of the test atmosphere was inserted.

Pressurized air was obtained from a compressor (type Dental 250, Jun Air, Nørresundby, Denmark). Water, grease and particles were removed from the pressurized air by passing through a set of filters (type FLA-20 equipped with elements A, B and D, Schumacher, Crailsheim, Germany). The flows of test material and air were regulated with flow controlers.

3.6.2 Characterization of the test atmosphere

Concentration measurement

During exposure, the actual concentration of test material was semi-continuously monitored (approx. 28 measurements/hour) using an infrared analyzer (Miran 80, Foxboro-Wilks, Norwalk, Conn., USA) which operated under the following conditions:

Wave numbers	: 892 cm^{-1} (HFC 143)
	1188 cm^{-1} (HFC 143)
	790 cm^{-1} (control of drift)
Optical path length	: 0.75 m
Slit width	: 1 mm

The actual concentration was measured during the whole exposure period. The mean concentration was calculated over the 330 minute period from 30 minutes after the start of the exposure period. Thirty minutes was assumed to be the time needed to reach a stable concentration under the given exposure conditions.

During the exposure the gas bottle was placed on a balance. The weight of the gas bottle, the flow of test material and the flow of pressurized air were recorded every 30 minutes. From the data on weight of test material used and the total flow through the exposure chamber (sum of the flow of test material and pressurized air) the nominal concentration was calculated.

Temperature and relative humidity.
During the connection of the animals to the exposure chamber the temperature and relative humidity of the atmosphere inside the chambers were measured using a Vaisala HMP 35 probe and a Vaisala HM32UT temperature and humidity indicator (Vaisala, Helsinki, Finland) and recorded with a chart recorder. Readings of the temperature and relative humidity made every 30 minutes were used to calculate mean values.

3.7 Biological variables investigated

3.7.1 Clinical signs and mortalities

On all exposure days the animals were observed to check for signs of ill-health or reaction to treatment twice daily; viz. just before placing the animals in the restraining tubes and within one hour after the end of the 6-hour exposure time. During weekends, when the animals were not exposed to HFC 143, observations were made once daily, in the morning. Decedents were removed from their cages and examined as indicated in sections 3.7.7 (necropsy) and 3.7.8 (histology).

3.7.2 Body weights

All animals were weighed on the day of allocation to treatment, 7 days prior to the start of exposure. Thereafter the animals selected for the study were weighed twice weekly (days -3, 1, 5, 8, 12 and 14), and on the day of necropsy (day 15; to determine autopsy body weights). Weighing on exposure days took place before the start of exposure. (The first day of exposure was designated day 1 of the study).

3.7.3 Food consumption

Food consumption was assessed for each interval between the days on which the animals were weighed from day 7 to day 14. The individual food intakes were calculated by subtracting the weight of food pot plus food left (including spillage) from the weight of food pot plus food offered.

Where extreme amounts and/or wetting of spillage resulted in unreliable data, the relevant food consumption was excluded from calculations.

3.7.4 Water consumption

Water consumption was assessed for each interval between the weigh days from day-7 to 14. The individual water consumptions were calculated by subtracting the weight of water bottle plus-water left from the weight of water bottle plus water offered.

3.7.5 Haematology and clinical chemistry

Blood sampling

Immediately after urine collection (day 14), from each (fasted; see 3.7.6.) animal about 0.9 ml of blood was taken by orbital puncture under light ether anaesthesia; 0.3 ml of blood was transferred to a tube containing 0.78 mg Na_2 EDTA and used for haematologic examinations; 0.6 ml of blood was transferred to a tube containing 12.5 I.U. Li-heparine, and was used for clinical chemistry.

In addition, at necropsy the animals in the study were exsanguinated by means of aorta-punction. About 3 ml of blood was collected in 3-ml tubes containing 45 USP-units Li-heparine. After centrifugation the plasma was deep frozen for testosterone and oestradiol plasma levels. The animals were further exsanguinated, but the remainder of the blood was deleted.

From the animals to be used for validation of the hormone assays, as much blood as possible was collected in 3 ml-tubes and frozen as indicated above.

Haematology

The following haematology parameters were measured: WBC, RBC, platelets, haemoglobin, PCV, MCV, MCH, MCHC and differential white blood cell count. Reticulocytes were to be counted only if abnormalities in the red blood parameters were found. Details on methodology are presented in Appendix 3, table 1.

Clinical chemistry

The following clinical chemistry parameters were measured: glucose, albumin, urea, creatinin, aspartate aminotransferase, (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), sodium (NA^+), potassium (K^+), calcium (Ca^+), inorganic phosphate, cholesterol, triglycerides, testosterone and oestradiol. Details on methodology presented in Appendix 3, table 2.

3.7.6 Urinalysis

Immediately after the end of the last exposure (day 14), the animals were placed individually in metabolism cages. Overnight, for a period of about 16 hours, the urine was collected in glass bottles, cooled on ice-water. During urine collection no food was supplied, but the animals had free access to water.
In order to avoid possible contamination of the urine with fluoride traces from any piece of equipment, before use, the metabolism cages and all related equipment was rinsed with 3 l of nitric acid in 60 l water.

The following urine parameters were measured quantitatively: volume, specific gravity, osmolality, creatinine, γ-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), N-acetyl-b-D-glucosaminidase (NAG), sodium (Na^+) potassium (K^+), fluoride (F^-); in addition pH, glucose, protein, ketone bodies, nitrite (NO_2^-), bilirubin, urobilinogen, blood and leucocytes were measured qualitatively by means of a urine strip. To relate enzyme activity to protein secretion, quantitative measurements were also performed. Details on methodology are presented in Appendix 3, table 3.

3.7.7 Necropsy

Three animals, numbers 2206, 2310 and 2414 died during the study; the remainder surviving until the scheduled necropsy date. The animals dying during the study were examined as described for the survivors below, with the exception that the organs were not weighed before fixation.

A macroscopic examination was performed on all surviving animals on the scheduled necropsy date, any abnormality found being recorded. The animals were killed as indicated in section 3.7.5 under "blood sampling". After examination of the external appearance of the animal, including eyes, ears, nose, mouth, urogenital area, tail and extremities and condition of the fur; the animals were opened by a single mid-line incision and the cervical tissues and the thoracic and abdominal contents were examined. The heart and thymus were carefully dissected away from the mediastinum and lungs and discarded after examination. The trachea was divided just below the thyroid cartilage, and the lung and trachea dissected away from the aorta and oesophagus. After removal the lungs were first weighed and then inflated with about 5 ml fixative, introduced through the trachea. The trachea was ligated and the inflated lungs were then placed into fixative.

The livers, adrenals and kidneys were carefully dissected free of fat, weighed and placed in the appropriate fixative. From the males, the testes were also freed from extraneous material and weighed. Subsequently one testis from each animal was fixed for histological examination; the other testis was frozen in liquid nitrogen and subsequently stored at -80°C for possible subsequent analysis of testosterone and oestradiol levels, if indicated by the results of the plasma hormone level analyses.

3.7.8 Histology

The fixatives used were:

Tissue	Fixative	Post-fixative
Lung with trachea, liver, kidney	10% phosphate buffered formalin with 2% (w/v) phenol	None
Adrenals	Müller	10% phosphate buffered formalin with 2% (w/v) phenol
Testis (one per male)	Bouin	None

After processing, the tissues were embedded in a paraffin-wax/ester-wax combination and cut at a nominal 5 µm. Sections from all tissues were stained with haematoxylin and eosin.

3.8 Statistics

The basic analysis consists of a two-way analysis of variance, followed by the Student's t-test. The means in the tables can differ from the raw means, when some values are missing. This is caused by the correction for blocks. If a studentized residual greater than 3 is found, Wilcoxon's two sample rank-sum test is used instead of the t-test.
In the summary tables the test results are denoted by:

- * difference from control significant, $p \leq 0.05$
- ** difference from control significant, $p \leq 0.01$
- W Wilcoxon and medians in stead of t-test and means

Ordinal data is summarized as frequency counts and it is tested with Somers'D statistic[1] whether the trend with increasing dose significantly differs from zero.

Ref. 1: Goodman, L.A. and Kruskal, W.H.; Measures of Association for Cross Classifications, IV: Simplification of Asymptotic Variances; JASA, 1972, 67, 415-421

3.9 Deviations from the protocol

The following deviations from the protocol have occurred:

- Almost throughout the study, room temperature ranged from 20-22°C, which is below the range indicated in the protocol (22-24°C). During two short intervals room temperature fell within the specified range, reaching maximum of 23°C. During one short interval the temperature dropped to 19°C.

- During one 28-hour interval, the relative humidity exceeded the range specified in the protocol (30-70%), reaching a maximum of 87%, due to technical problems. In addition on several days short peaks were recorded upto about 80%, coinciding with the cleaning of the animal room.

- On 7th June (day 5) the water bottles of male numbers 1415 and 2413 were noted to be empty. This means that these two animals had, most likely for a very short period of time, no access to drinking water.

- On 7th June (day 5) female numbers 1520 (control) and 2518 (test) erroneously have been interchanged in the exposure chambers for about four hours. Immediately after discovery the two animals were replaced to their respective groups/exposure chambers.

- Rinsing of the metabolism cages and related equipment was done with 3 l nitric acid in 60 l water, not in 20 l water, as indicated in the protocol. This was done to increase the volume so that pieces of equipment could be fully covered with the liquid.

- For an unknown period of time, there has been no overpressure in the animal room. As no indication of infections was found during clinical observations or at necropsy of the animals, this was not considered to have affected the results of the study.

- On the last day of exposure, observation of clinical signs was performed less detailed than on other days, when the animals were examined after they had been placed back to their cages. The reason was that the animals were placed in the metabolism cages immediately after removal from the restraint tubes, at the end of the exposure.

None of the above mentioned deviations are considered to have affected the integrity of the study.

4. RESULTS

4.1 Test atmosphere characterization (Tables 1,2)

The exposure concentrations were very close to the target concentration.
On all exposure days, the nominal concentration, calculated from the amount of test material used and the air flow through the exposure chamber, differed less than 3% from the target concentration (10,000 ppm). The daily mean actual concentrations based upon 173-175 recordings per day, never differed more than 2% from the target concentration. Daily means are presented in table 1.

The daily mean temperature in the exposure chamber during treatment ranged from 21.5 to 23.4°C; the daily mean relative humidity ranged from 34.3 to 39.0%. Daily means are presented in table 2.

4.2 Mortalities

Three female rats, all from the HFC 143 treated group died during the study. All three deaths were considered to be related to exposure with the test compound.

Female numbers 2206, 2414 and 2310 were found dead on days 3, 4 and 6 of the study, respectively. For animal numbers 2206 and 2310 the clinical signs recorded on the previous day or days confirmed that these animals were affected more than most of the other HFC 143-treated animals. For animal number 2414 this was not the case; this animal was unexpectedly found dead; the previous day was a non-exposure day, and no indication of a poor condition was noted previously for this animal before. Autopsy findings of all three animals indicated findings that were considered to be compound-related.

4.3 Clinical signs

In both sexes, the most frequently occurring clinical signs in the HFC 143-treated group were slightly decreased locomotor activity (mainly observed during the first week), slight ptosis (mainly observed after the second exposure, and in the males still present the next morning), abnormal posture and gait (mainly slight, but occasionally moderate to severe; mainly arched, but occasionally accompanied by high lower back and/or staggering; generally persisting almost throughout the study; the morning after the first exposure the females were slightly more affected than the males). In addition slightly reduced grooming activity was observed on the second exposure day (males only after exposure; females before and after exposure).

In the females, slight hypothermia was observed on the mornings after the first and second exposure days; dirt around the nose was observed before and after the second exposure as well as the next morning. In addition, both signs were recorded for female 2310, after the fourth exposure (day 6 of the study), the day before she died.
None of these signs were observed for any of the control animals. Details on the signs above are presented in Appendix 1, tables 1-1 through 1-7.

In addition, some infrequently occurring signs were recorded. From day 7 onwards a bald or dirty area in the neck was noted for control female 1311.
In the HFC 143-treated group, female 2208 showed flank respiration, reduced dermal blood supply and reduced body tone after the fourth exposure (day 6); female 2310, which died on day 7, showed reduced body tone after the third exposure (day 5) and on day 6 flank respiration (before and after exposure), reduced respiration rate (before exposure) and reduced dermal blood supply (after exposure); for females 2312 and 2416 sniffing was recorded on day 6 (before exposure); diarrhoea was noted for female 2518 on days 12 and 13 (after exposure).

4.4 Body-weight and weight-gain (Tables 3, 4; Appendix 1, tables 2, 3)

Exposure to HFC 143 adversely affected weight-gain in both sexes. Most HFC 143-treated animals lost weight during the first 4-day interval (including two non-exposure days), whereas the majority of the males and all females in the control group were gaining weight. With exception of the interval from days 8-12, weight-gain in the males was statistically significantly reduced during all intervals. Noteworthy is the marked - and statistically significant - increase in weight-gain from days 8-12 (including two non-exposure days) in both sexes. Despite this temporary increase, treated males had generally lost weight when taking into account the full 14-day study period, also resulting in a statistically significant reduction when compared to controls.
Body weight-gain of the surviving females was less severely affected. Statistically significant reductions were present at the intervals from days 1-5 and 12-14; as for the males a significant increase was present from days 8-12. Weight-gain during days 5-8 as well as overall weight-gain of the surviving females in the test group was comparable with the control group.

4.5 Food consumption (Table 5; appendix 1, table 4)

In the males, with exception of the interval from days 8-12, food consumption was statistically significantly reduced throughout the study, the effects being most marked during the first week. From days 8-12 food consumption was comparable with the control group.

In the females, food consumption was statistically significantly reduced during the intervals from days 1-5, 12-14 and 1-14. From days 5-12 food consumption was comparable with the control group.

4.6 Food conversion (Table 6)

In both sexes food conversion patterns followed the weight-gain patterns.

4.7 Water consumption (Table 7; appendix 1, table 5)

There was a large inter-animal variation in water consumption in both sexes, particularly in the females of the HFC 143-treated group. Throughout the study water consumption in the males was comparable in both groups. In the females, water consumption was markedly, and statistically significantly, increased from day 5 onwards.

4.8 Haematology (Table 8; appendix 1, table 6)

Haematological examinations showed slightly higher values in the red blood cell indices of the treatment groups of both males and females. Statistically significant differences were: MCV in both sexes, MCH in the males, and MCHC in the females.

At microscopic examination of the blood smears, a marked increase in the number of normoblasts was observed in both sexes, this effect being more pronounced in the females than in the males. In addition, in two females a significant number of Howell Jolly bodies was observed. Details are presented as footnotes in tables 6-1 and 6-2 in appendix 1.

White blood cell examinations showed a statistically significant reduction in the total white blood cell and absolute lymphocyte numbers in the males. In addition, statistically significantly increased percentages of neutrophils and eosinophils were present. The latter increases were a consequence of the reduction in lymphocytes. In the females, the relative and absolute number of eosinophils as well as the platelet counts were statistically significantly increased.

4.9 Clinical chemistry (Table 9; appendix 1, table 7)

The following statistically significant differences from control were present: in the males, decreased ALP and increased glucose, creatinine and cholesterol levels; in the females increased ALP and inorganic phosphate levels. No effects were found on the plasma oestradiol and testosterone levels.

4.10 Urinalysis (Table 10; appendix 1, table 8)

In the males, a statistically significant reduction was present in the excretion of sodium and protein (urine concentration and total excretion), and potassium and creatinine (total excretion only); fluoride concentration and total excretion were increased.

In the females the following statistically significant differences from control were observed: increased urine volume, fluoride concentration and total excretion, and total creatinine excretion; reduced alkaline phosphatase activity expressed per amount creatinine excreted, protein excretion and NAG activity.

4.11 Organ weights (Table 11; appendix 1, table 9)

In the males, testis weights were severely reduced in the HFC 143-treated group, both absolute and relative group mean weights being about 55% of that in the control group. In the females absolute and relative weights of adrenals and lungs were increased compared to the control females. The above indicated differences were all statistically significant. No other effects on organ weights were noted.

4.12 Gross Pathology (Table 12; appendix 1, table 10; appendix 2)

Three animals, numbers 2206, 2310 and 2414 died during the study, over a period of 3 to 6 (inclusive) days on the study; the remainder survived until the scheduled necropsy date.

Among the decedent rats, apart from non-specific indications of poor general condition, there was fluid in thoracic cavities and fluid or foam in the upper respiratory tracts. In addition, one animal had fluid in the abdominal cavity, and all decedents showed changes in the liver, including white areas or spots, roughened surface and a pronounced lobular pattern.
No treatment related macroscopic changes were seen at autopsy among the animals surviving to the end of the treatment period.

4.13 Microscopic observations (Table 13; appendix 1, table 11; appendix 2)

The three females dying during the study showed necrosis in the liver. The two animals dying earlier (nos. 2206 and 2414) had areas of necrotic liver tissue, whereas in the animal surviving longer (no. 2310) the necrosis was confined to the centrilobular regions. All three animals dying in the study showed changes in the lungs, characterized by congestion, oedema round vessels and localized areas of alveolar fibrosis.

All the animals surviving at the end of the study showed some degree of cellular alteration in the adrenal cortex; this was considered to be a stress-related phenomenon associated with the restraint required for the nose-only exposure in both control and treated animals. Minor changes were seen in the kidneys of both groups, but there were no treatment-related kidney findings. In contrast to the animals dying during the study there were no treatment-related changes observed in the livers of surviving rats.

In the males there was treatment-related fibrosis of the alveolar walls, mainly localized, but in two animals (nos. 2413 and 2415) this was generalized.

The incidence of pulmonary alveolar fibrosis was as follows:

Sex	MALE		FEMALE		
Dose group (ppm)	0	10000	0	10000	10000
	Terminal	Terminal	Terminal	Decedent	Terminal
Number examined:	10	10	10	3	7
Fibrosis					
- localized	0	5	3	2	1
- areas	0	1	0	0	0
- general	0	2	0	0	0

All testes from the treatment group showed atrophy of the tubules with degenerated spermatids. Spermatozoa were absent in 8 animals and greatly reduced in number in the remaining two. In 6 animals the seminiferous tubules contained giant cells.

No changes attributable to treatment were seen in the tracheas.

In view of the unexpectedly high testicular toxicity and of the absence of effects in plasma hormone levels, it was decided not to analyse the testicular hormone levels.

4.14 Fluoride intake and excretion

In view of the increased urinary fluoride excretion in both sexes, the excretion was roughly compared to the fluoride intake via the diet and drinking water.

In all four samples of drinking water analyzed, fluoride concentration was identical, viz. 0.10 mg/ml. From these results it is assumed that fluoride concentration in the drinking water was 0.10 mg/ml for all animals throughout the study. The dietary concentration of fluoride was 23 mg/kg (see appendix 4).

The following table presents some data on fluoride intake and excretion:

	Mean daily fluoride intake				Urinary fluoride excretion at day 14	
Group	Food [a]	Water [b]	Food		Water	
	(mg)	(mg)	(mg)	(% of control)	(µ mol)	(% of control)
MALES						
Control	0.592	4.105	4.697		25.8	
10000 ppm	0.518	4.242	4.760	101.3	64.5	250
FEMALES						
Control	0.460	3.366	3.826		13.9	
10000 ppm	0.345	4.703	5.048	131.9	49.3	355

[a] = mean food consumption (days 1-14) x 23/1000 mg.
[b] = mean water consumption (days 1-14) x 0.10 mg.

The results clearly indicate that the increase in fluoride excretion in the HFC 143-treated animals cannot be explained only by the increase in fluoride intake via diet and drinking water. The results further suggest that the majority of the fluoride excreted is originating from the test compound.

5. DISCUSSION

The three mortalities (all females) were considered to be treatment-related. The actual cause of death could not be assessed. Although the liver necrosis is considered to be treatment-related, a causal relationship with the interval between death and necropsy of the animals cannot be excluded.
Treatment-related clinical signs were observed, the females being slightly more affected than the males. The signs observed are mainly indicative of central nervous system and locomotor disturbances.

With respect to body weight and food consumption, the females seemed to be slightly more affected than the males. Water consumption was not affected in the males, but was increased in the females.

The minor differences with control in the red blood cell indices are related to the high number of normoblasts observed. Although RBC, Hb and PCV were not affected, these changes are considered to be indicative of an effect on erythropoeisis.
Total white blood cell counts were relatively high in the male control group when compared to historical background data. This increase is caused by an increase in both neutrophils and lymphocytes. Despite this, the percentage of lymphocytes in the control group is within normal limits. Therefore, the decrease in absolute and relative lymphocyte counts in the HFC 143-treated group is considered to be compound-related.
As the number of platelets counted was well within the normal biological range, the increased platelet count in the treatment group of the females is considered to be of no toxicological significance.

The slight changes seen in plasma alkaline phosphatase are, although probably related to treatment, considered to be of minor toxicological relevance. A different response was observed between males and females, the males showing a decrease, the females an increase. As food consumption, growth and various organs or organ systems can influence plasma ALP levels, no further conclusion can be drawn. It should, however, be noted that total ALP plasma activity measurement is generally accepted as a sensitive non-target-organ-specific parameter.
The minor changes in plasma glucose and cholesterol levels in the males are probably due to slight changes in metabolism. As all values fell within the normal range, these effects are considered to be of no toxicological relevance.
Increased plasma inorganic phosphate level in the treated females is considered to be treatment-related.

The large variation in oestradiol concentrations is probably related to the oestrous cycle stages. From the literature (1) it is known that oestradiol levels can vary from less than 10 ng/l (basic level, at oestrous) up to about 50 ng/l (peak level, at pro-oestrous).

The slight decrease in sodium and potassium excretion in the males is probably due to slight alterations in electrolyte balance. This minor change is considered to be of no toxicological importance. The reduced urinary protein excretion in the male treatment group might be caused by a reduction in testicular protein secretion.
In the HFC 143-treated females an increased urine volume was associated with a higher water intake. Other slight changes seen in the urine parameters of the females were considered to be secondary to the increased urine voidence.

The increase of urinary fluoride excretion is expected to result mainly from metabolism of HFC 143. If a similar pattern occurs in men, urinary fluoride excretion might well be a good parameter for bio-monitoring purposes.

The most serious histopathological effect was testicular tubular atrophy, with degeneration of the spermatids and the presence of giant cells, but with Leydig and Sertoli cells appearing normal. These findings are indicative of a disturbance of the spermatogenesis, most likely by affecting late spermatids and/or early spermatocytes. Due to the design of the present study, it is unpredictable whether this effect would be reversible, whether it also occurs after single exposure, or what the NOEL for this effect would be after single or multiple exposure(s).
Other target organs identified were liver (decedents only), adrenals (based upon weights only) and lungs (based upon weights as well as microscopy).

6. CONCLUSIONS

From the results of this study the following conclusions were drawn:

- HFC 143, when given to Sprague Dawley rats by the inhalatory route at 10,000 ppm for 2 weeks, 5 days per week, 6 hours per day, resulted in clear toxicity. Three females died within 6 days with clinical signs comparable to other treated females. The major target organ appeared to be the testis. Other target organs identified are the lungs and possibly the adrenals. Most relevant other parameters affected were clinical signs; body weight and food consumption (reduced); increased water consumption associated with increased urine volume (in the females) and lymphocytes (decreased in males), and effects on the erythropoeitic system.

- For future repeated dose studies, dose levels higher than 10,000 ppm are not recommended. In view of the severe testis effects, no recommendation for low dose levels can be given, as no estimation of a NOEL can be made.

- It is recommended to investigate whether the testis effects also occur after single exposure, whether these effects are reversible (after single exposure and, if so, also after repeated exposure) and to assess the NOEL for this effect.

- Urinary fluoride excretion might well be a useful parameter for bio-monitoring purposes.

7. REFERENCE

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Table 1: Mean daily concentration of HFC 143 in the exposure chamber in the treatment group.

Date	Day in study	Concentration (ppm)		
		Nominal	Actual (sd)	n
June 3, 1993	1	9942	10046 (146)	173
June 4, 1993	2	9942	10048 (53)	173
June 7, 1993	5	10020	10051 (261)	174
June 8, 1993	6	9942	10048 (40)	175
June 9, 1993	7	9864	10023 (136)	175
June 10, 1993	8	10175	10190 (222)	175
June 11, 1993	9	9864	10082 (134)	178
June 14, 1993	12	10097	9962 (156)	175
June 15, 1993	13	9864	10029 (90)	175
June 16, 1993	14	9709	9853 (94)	175

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Table 2: Mean temperature (t; °C) and relative humidity (RH; %) in the exposure chamber

Date	Day in study	t (sd)	RH (sd)	n
CONTROL				
June 3, 1993	1	21.7 (0.52)	38.8 (6.27)	13
June 4, 1993	2	21.7 (0.31)	34.3 (6.78)	13
June 7, 1993	5	22.0 (0.32)	38.4 (4.34)	13
June 8, 1993	6	21.7 (0.38)	37.5 (4.51)	13
June 9, 1993	7	22.4 (0.17)	36.9 (5.86)	13
June 10, 1993	8	23.4 (0.60)	36.1 (2.76)	13
June 11, 1993	9	23.0 (0.37)	36.6 (3.61)	13
June 14, 1993	12	22.2 (0.36)	36.6 (5.18)	13
June 15, 1993	13	22.0 (0.55)	36.6 (4.60)	13
June 16, 1993	14	22.1 (0.42)	39.0 (5.24)	13
HFC 143				
June 3, 1993	1	21.8 (0.56)	39.1 (6.76)	13
June 4, 1993	2	21.8 (0.23)	34.6 (6.89)	13
June 7, 1993	5	22.0 (0.33)	38.3 (5.12)	13
June 8, 1993	6	21.5 (0.45)	37.9 (4.63)	13
June 9, 1993	7	22.3 (0.43)	37.3 (5.68)	13
June 10, 1993	8	23.3 (0.66)	35.2 (3.75)	13
June 11, 1993	9	22.9 (0.52)	36.6 (3.84)	13
June 14, 1993	12	22.2 (0.46)	36.6 (5.15)	13
June 15, 1993	13	22.0 (0.59)	36.7 (4.18)	13
June 16, 1993	14	22.1 (0.44)	38.1 (4.89)	13

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Table 3: MEAN BODY WEIGHTS -- gram

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
-7		337.8	(14.5)	335.9	(15.4)	
-3		369.7	(17.6)	371.4	(17.7)	
1		390.7	(17.9)	394.5	(20.2)	
5		394.1	(18.8)	380.8	(25.3)	
8		392.5	(18.5)	373.7	(23.4)	
12		402.8	(19.8)	397.7	(18.2)	
14		403.4	(20.5)	389.2	(19.1)	
FEMALES						
-7		253.8	(5.9)	253.0	(6.5)	
-3		270.1	(8.9)	266.1	(10.4)	
1	W	272.0	(11.1)	267.0	(9.7)	
5		277.7	(12.0)	263.5	(12.8)	*
8		275.6	(10.6)	268.8	(13.0)	
12		280.1	(12.7)	287.1	(8.2)	
14		283.1	(12.3)	278.4	(11.7)	

Table 4: MEAN DAILY BODY WEIGHT-GAINS -- gram

	Group (ppm) 0		10000		
	mean	(sd)	mean	(sd)	
MALES					
-7...1	7.57	(1.89)	8.40	(1.48)	
1...5	0.95	(1.59)	-3.40	(3.96)	**
5...8	-0.57	(2.51)	-2.40	(2.06)	*
8...12	2.55	(0.99)	6.00	(2.40)	**
12...14	0.25	(2.55)	-4.40	(2.46)	**
1...14	0.98	(1.29)	-0.43	(0.94)	*
FEMALES					
-7...1	2.61	(1.22)	2.27	(1.03)	
1...5	1.43	(1.14)	-1.02	(2.04)	*
5...8	-0.73	(1.57)	0.60	(2.75)	
8...12	1.20	(0.86)	4.51	(2.52)	**
12...14	1.50	(2.69)	-4.25	(4.05)	**
1...14	0.87	(0.58)	0.68	(1.08)	

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

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Table 5 : MEAN FOOD CONSUMPTION -- gram/day

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
-7...1		31.07	(2.61)	32.53	(3.67)	
1...5		27.48	(2.30)	20.25	(5.82)	**
5...8		23.33	(2.40)	19.90	(3.03)	*
8...12		26.28	(1.67)	26.70	(1.96)	
12...14		24.75	(1.80)	22.55	(1.66)	*
1...14		25.73	(1.71)	22.51	(2.53)	**
FEMALES						
-7...1		23.54	(1.94)	22.50	(1.76)	
1...5		22.05	(2.18)	14.17	(5.68)	**
5...8		18.63	(1.99)	16.54	(4.52)	
8...12		20.53	(1.63)	20.99	(1.46)	
12...14		21.25	(2.12)	18.44	(1.58)	*
1...14		19.98	(1.94)	15.00	(2.90)	**

Table 6: MEAN FOOD CONVERSION -- ratio

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
-7...1		0.241	(0.046)	0.258	(0.032)	
1...5	W	0.019	(0.057)	-0.104	(0.395)	**
5...8		-0.031	(0.108)	-0.131	(0.124)	*
8...12		0.097	(0.036)	0.224	(0.084)	**
12...14		0.007	(0.099)	-0.199	(0.122)	**
1...14		0.037	(0.047)	-0.021	(0.044)	*
FEMALES						
-7...1		0.110	(0.050)	0.099	(0.046)	
1...5	W	0.049	(0.049)	-0.064	(0.445)	*
5...8		-0.042	(0.088)	-0.001	(0.191)	
8...12		0.048	(0.037)	0.206	(0.133)	*
12...14		0.071	(0.126)	-0.222	(0.210)	**
1...14		0.044	(0.029)	0.038	(0.085)	

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

Table 7: MEAN WATER CONSUMPTION -- gram/day

	Group (ppm) 0		10000		
	mean	(sd)	mean	(sd)	
MALES					
-7...1	45.51	(16.01)	44.11	(13.26)	
1...5	43.40	(13.64)	40.38	(14.95)	
5...8	38.43	(8.86)	44.83	(19.46)	
8...12	40.98	(9.17)	44.25	(13.79)	
12...14	40.45	(8.60)	39.25	(8.57)	
1...14	41.05	(10.03)	42.42	(13.55)	
FEMALES					
-7...1	31.46	(4.78)	28.86	(4.36)	
1...5	34.83	(7.78)	34.68	(17.56)	
5...8	30.87	(6.29)	49.21	(19.92)	*
8...12	34.03	(6.30)	51.60	(13.72)	**
12...14	34.80	(5.81)	50.68	(14.14)	*
1...14	33.66	(5.91)	47.03	(13.65)	*

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

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Table 8: MEAN HAEMATOLOGY DATA

	Group (ppm) 0 mean	(sd)	10000 mean	(sd)	
MALES					
RBC 10^{12}/l	8.621	(0.490)	8.248	(0.527)	
HGB mmol/l	10.54	(0.42)	10.59	(0.67)	
HCT 1/1	0.4655	(0.0227)	0.4684	(0.0256)	
MCV fl	54.04	(1.19)	56.83	(1.86)	** ↑
MCH fmol	1.2244	(0.0484)	1.2846	(0.0416)	** ↑
MCHC mmol/l	22.67	(0.66)	22.61	(0.56)	
PLT 10^9/l	W 1027.5	(73.2)	961.5	(250.6)	
WBC 10^9/l	15.72	(3.75)	7.21	(1.57)	** ↓
Neutro %	14.7	(8.5)	26.5	(3.9)	** ↑
Lymph %	82.9	(8.5)	70.0	(4.4)	** ↓
Mono %	1.6	(1.4)	1.6	(1.6)	
Eosin %	0.8	(1.0)	1.9	(1.1)	* ↑
Neutro 10^9/l	2.383	(1.571)	1.886	(0.379)	
Lymph 10^9/l	12.964	(3.095)	5.065	(1.210)	** ↓
Mono 10^9/l	0.243	(0.262)	0.127	(0.135)	
Eosin 10^9/l	0.131	(0.162)	0.136	(0.082)	
FEMALES					
RBC 10^{12}/l	7.764	(0.276)	7.474	(0.532)	
HGB mmol/l	10.08	(0.28)	9.81	(0.60)	
HCT 1/1	0.4355	(0.0103)	0.4337	(0.0207)	
MCV fl	56.12	(1.45)	58.13	(1.95)	* ↑
MCH fmol	1.2991	(0.0361)	1.3137	(0.0218)	
MCHC mmol/l	23.15	(0.46)	22.60	(0.50)	* ↓
PLT 10^9/l	970.8	(64.7)	1115.3	(155.3)	* ↑
WBC 10^9/l	7.15	(2.00)	5.95	(2.34)	
Neutro %	18.1	(7.4)	21.2	(9.9)	
Lymph %	79.9	(7.5)	74.7	(9.7)	
Mono %	1.2	(0.9)	1.2	(1.5)	
Eosin %	0.8	(1.0)	2.8	(2.1)	** ↑
Neutro 10^9/l	1.274	(0.526)	1.383	(1.213)	
Lymph 10^9/l	5.727	(1.742)	4.365	(1.529)	
Mono 10^9/l	0.092	(0.099)	0.056	(0.059)	
Eosin 10^9/l	0.058	(0.073)	0.141	(0.079)	* ↑

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

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Table 8: MEAN HAEMATOLOGY DATA

	Group (ppm) 0 mean	(sd)	10000 mean	(sd)	
MALES					
RBC 10^{12}/l	8.621	(0.490)	8.248	(0.527)	
HGB mmol/l	10.54	(0.42)	10.59	(0.67)	
HCT 1/1	0.4655	(0.0227)	0.4684	(0.0256)	
MCV fl	54.04	(1.19)	56.83	(1.86)	**
MCH fmol	1.2244	(0.0484)	1.2846	(0.0416)	**
MCHC mmol/l	22.67	(0.66)	22.61	(0.56)	
PLT 10^9/l	W 1027.5	(73.2)	961.5	(250.6)	
WBC 10^9/l	15.72	(3.75)	7.21	(1.57)	**
Neutro %	14.7	(8.5)	26.5	(3.9)	**
Lymph %	82.9	(8.5)	70.0	(4.4)	**
Mono %	1.6	(1.4)	1.6	(1.6)	
Eosin %	0.8	(1.0)	1.9	(1.1)	*
Neutro 10^9/l	2.383	(1.571)	1.886	(0.379)	
Lymph 10^9/l	12.964	(3.095)	5.065	(1.210)	**
Mono 10^9/l	0.243	(0.262)	0.127	(0.135)	
Eosin 10^9/l	0.131	(0.162)	0.136	(0.082)	
FEMALES					
RBC 10^{12}/l	7.764	(0.276)	7.474	(0.532)	
HGB mmol/l	10.08	(0.28)	9.81	(0.60)	
HCT 1/1	0.4355	(0.0103)	0.4337	(0.0207)	
MCV fl	56.12	(1.45)	58.13	(1.95)	*
MCH fmol	1.2991	(0.0361)	1.3137	(0.0218)	
MCHC mmol/l	23.15	(0.46)	22.60	(0.50)	*
PLT 10^9/l	970.8	(64.7)	1115.3	(155.3)	*
WBC 10^9/l	7.15	(2.00)	5.95	(2.34)	
Neutro %	18.1	(7.4)	21.2	(9.9)	
Lymph %	79.9	(7.5)	74.7	(9.7)	
Mono %	1.2	(0.9)	1.2	(1.5)	
Eosin %	0.8	(1.0)	2.8	(2.1)	**
Neutro 10^9/l	1.274	(0.526)	1.383	(1.213)	
Lymph 10^9/l	5.727	(1.742)	4.365	(1.529)	
Mono 10^9/l	0.092	(0.099)	0.056	(0.059)	
Eosin 10^9/l	0.058	(0.073)	0.141	(0.079)	*

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

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Table 9: MEAN CLINICAL CHEMISTRY DATA

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
AST U/L	W	68.0	(12.6)	70.5	(128.1)	
ALT U/L	W	30.0	(4.9)	24.5	(14.9)	
ALP U/L		298.1	(59.5)	230.9	(31.6)	**
Gluc mmol/l		5.746	(0.728)	6.595	(1.014)	*
Album g/l		44.02	(2.44)	43.36	(3.39)	
Urea mmol/l		4.759	(0.585)	4.968	(0.872)	
Creat µmol/l		40.7	(3.8)	45.7	(5.1)	*
Chol mmol/l		1.059	(0.132)	1.577	(0.369)	**
Trig mmol/l		0.521	(0.132)	0.525	(0.167)	
Oestradiol ng/l		0.621	(0.229)	0.542	(0.188)	
Testosteron ng/l		1768.9	(802.4)	1552.5	(863.0)	
Ca++ mmol/l		2.596	(0.036)	2.614	(0.109)	
In.pho mmol/l	W	2.410	(0.148)	2.405	(0.403)	
Na+ mmol/l		146.6	(1.2)	145.8	(1.9)	
K+ mmol/l		3.725	(0.372)	3.486	(0.129)	
FEMALES						
AST U/L		54.3	(15.8)	70.4	(24.5)	
ALT U/L		25.8	(3.5)	22.8	(2.7)	
ALP U/L		138.7	(29.7)	169.6	(23.4)	*
Gluc mmol/l		7.414	(0.862)	7.228	(1.414)	
Album g/l		48.91	(2.23)	47.27	(2.41)	
Urea mmol/l		5.678	(0.915)	4.994	(0.547)	
Creat µmol/l		52.0	(5.9)	51.0	(6.3)	
Chol mmol/l		1.940	(0.603)	1.733	(0.277)	
Trig mmol/l		0.429	(0.141)	0.516	(0.137)	
Oestradiol ng/l		16.759	(15.774)	5.164	(4.995)	
Testosteron ng/l		121.4	(82.9)	108.0	(43.3)	
Ca++ mmol/l		2.620	(0.054)	2.620	(0.097)	
In.pho mmol/l		1.811	(0.225)	2.279	(0.356)	*
Na+ mmol/l		145.5	(1.3)	144.1	(2.4)	
K+ mmol/l		3.643	(0.389)	3.589	(0.307)	

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

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Table 10-1: MEAN URINE ANALYSIS DATA - Numeric data

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
Vol ml		24.47	(11.00)	27.68	(11.35)	
S.G.		1.0176	(0.0068)	1.0130	(0.0058)	
Osmo Osm/kg		0.5852	(0.2406)	0.4484	(0.1903)	
Na+ mmol/l		24.84	(15.76)	12.23	(6.53)	*
K+ mmol/l		62.46	(32.36)	40.91	(22.56)	
Na+ mmol		0.535	(0.238)	0.298	(0.165)	*
K+ mmol		1.281	(0.368)	0.920	(0.195)	*
F- mg/l		1.175	(0.461)	2.905	(1.802)	**
F- µmol		25.8	(7.6)	64.5	(17.4)	**
ALP U/l		74.33	(68.22)	29.86	(58.56)	
ALP U/mmol Cr		11.69	(7.02)	5.58	(8.55)	
ALP U/g Prot		67.9	(227.6)	218.8	(301.8)	
GGT U/l		686.3	(284.0)	677.9	(523.4)	
GGT U/mmol Cr		125.1	(41.6)	152.3	(62.6)	
GGT U/g Prot		4312.4	(2383.8)	6321.2	(2314.1)	
Creat µmol/l		5621.2	(2257.2)	4083.1	(1908.2)	
Creat µmol		118.1	(22.4)	94.3	(10.3)	**
Prot mg/l	W	158.0	(189.0)	78.5	(47.2)	*
Prot mg	W	3.200	(1.257)	2.235	(0.364)	**
NAG U/l		8.58	(1.80)	6.63	(2.91)	
NAG U/mmol Cr		1.65	(0.40)	1.66	(0.32)	
NAG U/g Prot		55.5	(18.6)	69.8	(13.4)	
FEMALES						
Vol ml		13.21	(7.44)	28.68	(16.54)	*
S.G.		1.0200	(0.0106)	1.0123	(0.0075)	
Osmo Osm/kg		0.6966	(0.4228)	0.4287	(0.2501)	
Na+ mmol/l		20.67	(21.17)	15.50	(12.54)	
K+ mmol/l		69.78	(45.45)	40.78	(26.35)	
Na+ mmol		0.167	(0.086)	0.313	(0.232)	
K+ mmol		0.666	(0.102)	0.757	(0.150)	
F- mg/l		1.275	(0.519)	2.057	(1.030)	*
F- µmol		13.9	(4.4)	49.3	(17.5)	**
ALP U/l		52.71	(63.92)	14.91	(11.93)	
ALP U/mmol Cr		6.67	(4.55)	2.67	(1.42)	*
ALP U/g Prot		282.4	(212.5)	129.5	(92.1)	
GGT U/l		322.1	(280.3)	232.3	(214.3)	
GGT U/mmol Cr		47.3	(16.8)	49.2	(27.0)	
GGT U/g Prot		2021.2	(889.8)	2377.2	(1761.7)	
Creat µmol/l		6265.6	(3750.9)	3958.4	(2619.0)	
Creat µmol		61.4	(8.6)	73.1	(5.1)	*
Prot mg/l		146.4	(73.2)	79.9	(26.0)	*
Prot mg		1.508	(0.343)	1.834	(0.844)	
NAG U/l		11.63	(6.04)	6.61	(2.79)	*
NAG U/mmol Cr		1.95	(0.30)	1.85	(0.44)	
NAG U/g Prot		80.0	(14.0)	81.8	(27.8)	

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

Table 10-2: MEAN URINE ANALYSIS DATA - Urine test strips

MALES

			Group (ppm)	
			0	10000
pH 5-9	**	6	3	10
		7	7	0
LEU -/++	**	-	3	8
		(+)	6	2
		++	1	0
NIT -/(+)		-	9	10
		(+)	1	0
PRO -/+++		-	5	7
		(+)	4	3
		+	1	0
GLU -/+++		-	10	10
KET -/++		-	9	10
		(+)	1	0
UBG -/+++		-	10	10
BIL -/+++		-	10	10
ERY -/+++		-	2	3
		(+)	5	7
		+	2	0
		+++	1	0

* Somers'D significant, $p \leq 0.05$
** Somers'D significant, $p \leq 0.01$

Table 10-3: MEAN URINE ANALYSIS DATA - Urine test strips

FEMALES

			Group (ppm)	
			0	10000
pH 5-9		5	0	1
		6	7	5
		7	3	1
LEU -/++	**	-	6	7
		(+)	4	0
NIT -/(+)		-	10	6
		(+)	0	1
PRO -/+++		-	9	6
		(+)	0	1
		+	1	0
GLU -/+++		-	10	7
KET -/++		-	9	7
		(+)	1	0
UBG -/+++		-	9	7
		(+)	1	0
BIL -/+++		-	9	7
		(+)	1	0
ERY -/+++		-	8	7
		(+)	2	0

* Somers'D significant, $p \leq 0.05$
** Somers'D significant, $p \leq 0.01$

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Table 11: MEAN ORGAN WEIGHTS -- absolute and relative to body weight

		Group (ppm) 0		10000		
		mean	(sd)	mean	(sd)	
MALES						
Body weight (g)		373.8	(18.3)	359.4	(17.7)	
Adrenal (mg)		70.7	(8.3)	69.0	(11.2)	
Adrenal rel		18.91	(2.00)	19.17	(2.66)	
Kidney (g)		2.647	(0.229)	2.535	(0.238)	
Kidney rel		0.707	(0.047)	0.706	(0.067)	
Liver (g)		9.574	(0.861)	9.765	(0.879)	
Liver rel		2.560	(0.175)	2.716	(0.182)	
Lung (g)	W	1.560	(0.187)	1.470	(0.740)	
Lung rel		0.4265	(0.0624)	0.4985	(0.1863)	
Testes (g)		3.367	(0.247)	1.795	(0.143)	**
Testes rel		0.901	(0.075)	0.500	(0.035)	**
FEMALES						
Body weight (g)		257.5	(10.9)	259.2	(9.1)	
Adrenal (mg)		78.3	(7.4)	91.8	(16.1)	*
Adrenal rel		30.43	(2.93)	35.30	(5.27)	*
Kidney (g)		1.770	(0.181)	1.682	(0.097)	
Kidney rel		0.686	(0.060)	0.649	(0.035)	
Liver (g)		7.137	(0.760)	7.367	(1.162)	
Liver rel		2.768	(0.228)	2.845	(0.445)	
Lung (g)		1.273	(0.061)	1.502	(0.235)	*
Lung rel		0.4940	(0.0264)	0.5827	(0.1095)	*

* difference from control significant, $p \leq 0.05$
** difference from control significant, $p \leq 0.01$
W Wilcoxon and medians instead of t-test and means

Table 12-1 SUMMARY OF MACROSCOPIC OBSERVATIONS -- Males

		Dose group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Kidney	pelvis slightly dilated	1	2
Lung	a few red spots	2	1
Skin	dark spots	0	1
	superficial lesions	1	0
Thymus	maculate	2	0

Table 12-2 SUMMARY OF MACROSCOPIC OBSERVATIONS -- Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Abdominal cavity	clear red fluid	0	1	0
Eye	diffuse white area	0	1	0
Genital area	fur stained	0	2	0
Heart	atrium dilated with blood	0	1	0
Liver	rough surface	0	2	0
	white areas	0	1	0
	white spot(s)	0	1	0
	lobular pattern pronounced	0	1	0
Lung	a few red spots	0	0	1
	diffuse red	0	2	0
Nose	clear red fluid	0	1	0
Skin	crust	0	2	0
Thoracic cavity	clear red fluid	0	1	0
	contains clear fluid	0	2	0
Trachea	clear red fluid	0	1	0
	contents foamy	0	2	0
Thymus	maculate	0	1	1
	red spots	0	2	0

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Table 13-1 SUMMARY OF MICROSCOPIC OBSERVATIONS -- Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Adrenal	Number examined:	10	10
	ectopic chromaffin tissue	1	1
	zona fasciculata, foci of cellular alteration		
	- slight	0	1
	- moderate	3	1
	- marked	3	2
	cortex areas of cellular alteration		
	- moderate	0	1
	- marked	4	4
Kidney	Number examined:	10	10
	no abnormalities detected	7	4
	pyelitis	0	1
	areas of cortical tubular necrosis	0	1
	areas of interstitial nephritis	0	1
	cortico-medullary junction		
	- cyst	1	0
	- casts	0	1
	- tubules dilated	0	1
	cortical scar	1	0
	localized area of basophilic cortical tubules	2	0
	subcapsular cyst	1	0
	slight unilateral hydronephrosis	1	1
	slight bilateral hydronephrosis	0	1

Table 13-1 SUMMARY OF MICROSCOPIC OBSERVATIONS -- Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Liver	Number examined:	10	10
	no abnormalities detected	9	9
	small foci of mononuclear cells		
	- a few	0	1
	- several	1	0
Lung	Number examined:	10	10
	no abnormalities detected	5	2
	areas of congestion	2	2
	congested	0	1
	localized area of fibrosis	0	5
	areas of fibrosis	0	1
	fibrosis	0	2
	alveolar wall slightly thickened	1	0
	slight proliferation of lymphoid tissue	1	0
	localized area of emphysema	1	0
	areas of emphysema	0	1

Table 13-1 SUMMARY OF MICROSCOPIC OBSERVATIONS – Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Testis	Number examined:	10	10
	no abnormalities detected	10	0
	sperm cells reduced		
	- marked	0	1
	- severe	0	1
	sperm cells not present	0	8
	spermatids degenerated	0	10
	atrophy of tubules	0	10
	giant cells in tubules	0	6
Trachea	Number examined:	10	10
	no abnormalities detected	9	10
	slight inflammation in submucosa	1	0

Table 13-2 SUMMARY OF MICROSCOPIC OBSERVATIONS -- Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Adrenal	Number examined:	10	3	7
	no abnormalities detected	0	1	0
	zona fasciculata, foci of cellular alteration			
	- slight	1	0	0
	- moderate	0	1	3
	- marked	4	1	0
	cortex areas of marked cellular alteration	5	0	4
Kidney	Number examined:	10	3	7
	no abnormalities detected	10	2	7
	slight dilatation of convoluted tubules	0	1	0
Liver	Number examined:	10	3	7
	no abnormalities detected	9	0	7
	areas of congestion	0	1	0
	a few small foci of mononuclear cells	1	0	0

Table 13-2 SUMMARY OF MICROSCOPIC OBSERVATIONS - Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Liver	areas of necrosis	0	2	0
	centrilobular parenchyma cells			
	contain small vacuoles	0	2	0
	necrosis	0	1	0
	swollen	0	1	0
Lung	Number examined:	10	3	7
	no abnormalities detected	6	0	3
	areas of congestion	1	2	1
	alveolar wall thickened			
	- slight	1	0	0
	- moderate	0	1	1
	vessel oedema			
	- marginal	0	0	1
	- moderate	0	1	0
	- marked	0	1	0
	- severe	0	1	0
	localized area fibrosis	3	2	1
Trachea	Number examined:	10	3	7
	no abnormalities detected	10	3	6
	slight inflammation of submucosa	0	0	1

Table 1-1 Clinical symptoms -- **Decreased locomotor activity**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2103	.	-	-	1	1	.	-	.	-	1	-	-	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2205	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2207	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2309	.	-	-	1	1	.	-	.	-	1	-	-	1	1	-	1	-	1	-	.	-	.	-	-	-	-	-	-	-	-
2311	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2413	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2415	.	-	-	1	1	.	-	.	-	1	-	-	1	1	-	1	-	1	-	.	-	.	-	-	-	-	-	-	-	-
2517	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2519	.	-	-	1	1	.	-	.	-	1	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
FEMALES																														
2102	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2104	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	-	-	.	-	.	-	1	-	-	-	-	-	-
2206	.	1	1	2	dead																									
2208	.	1	1	1	1	.	-	.	-	1	-	1	1	1	-	1	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2310	.	1	1	1	1	.	-	.	-	1	-	1	dead																	
2312	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2414	.	1	1	1	1	.	-	.	dead																					
2416	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2518	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-
2520	.	1	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined

Table 1-2 Clinical symptoms -- **Ptosis**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2103	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2205	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2207	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2309	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-
2311	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2413	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2415	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2517	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2519	.	-	-	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
FEMALES																														
2102	.	-	-	1	-	.	-	.	-	-	-	-	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2104	.	-	-	1	-	.	-	.	-	-	-	-	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2206	.	-	-	-	dead																									
2208	.	-	-	1	-	.	-	.	-	-	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2310	.	-	-	1	-	.	-	.	-	-	-	1	dead																	
2312	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2414	.	-	-	-	-	.	-	.	dead																					
2416	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2518	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2520	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined

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Table 1-3 Clinical symptoms -- **Abnormal posture (arched)**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2103	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
2205	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	1	-	1	-	.	-	.	-	-	-	-	-	-		
2207	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
2309	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	2	1	2	1	.	-	.	-	1	-	1	-	-		
2311	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	-	-	1	-	.	-	.	-	-	-	-	-	-		
2413	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2415	.	-	-	-	1	.	-	.	-	1	-	1	1	2	1	2	1	2	1	.	-	.	-	1	-	1	-	-		
2517	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
2519	.	-	-	-	-	.	-	.	-	1	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
FEMALES																														
2102	.	-	2	1	1	.	-	.	-	1	-	1	1	1	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2104	.	-	2	1	1	.	-	.	-	1	-	1	-	1	-	1	-	2	-	.	-	.	-	2	-	-	-	-		
2206	.	-	2	2	dead																									
2208	.	-	2	1	1	.	-	.	-	1	-	2h	1	1	-	2	-	1	-	.	-	.	-	-	-	-	-	-		
2310	.	-	2	1	1	.	1	.	-	2	2h	2	dead																	
2312	.	-	2	1	1	.	-	.	-	1	-	1	-	1	-	1	-	1	-	.	-	.	-	-	-	-	-	-		
2414	.	-	2	1	1	.	-	.	dead																					
2416	.	-	2	1	1	.	-	.	-	1	-	1	-	-	-	1	-	-	-	.	-	.	-	-	-	-	-	-		
2518	.	-	2	1	1	.	-	.	-	1	-	1	-	1	-	2	1	2	1	.	-	.	-	-	-	2	1	-		
2520	.	-	2	1	1	.	-	.	-	1	-	1	-	-	-	1	-	-	-	.	-	.	-	-	-	-	-	-		

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined h = + lower back high

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Table 1-4 Clinical symptoms -- **Abnormal gait (arched)**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	-	-	.	-	.	-	1h	-	1	-	-	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2103	.	-	-	-	-	.	-	.	-	1h	-	1	-	-	-	-	-	1	-	.	-	.	-	-	-	1	-	-		
2205	.	-	-	-	-	.	-	.	-	1h	-	1	-	-	-	1	-	1	-	.	-	.	-	-	-	-	-	-		
2207	.	-	-	-	-	.	-	.	-	1h	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
2309	.	-	-	-	-	.	-	.	-	1h	-	1	-	1	-	2	1	2s	1	.	-	.	-	1s	-	1s	-	-		
2311		-	-	-	-	.	-	.	-	1h	-	1	-	-	-	-	-	1	-	.	-	.	-	-	-	-	-	-		
2413		-	-	-	-	.	-	.	-	1h	-	1	-	-	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2415		-	-	-	1h	.	-	.	-	1h	-	1	1	2	1	2	1	2s	1	.	1	.	-	2	-	1	-	-		
2517		-	-	-	-	.	-	.	-	1h	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
2519	.	-	-	-	-	.	-	.	-	1h	-	1	-	1	-	-	-	-	-	.	-	.	-	-	-	-	-	-		
FEMALES																														
2102	.	-	1	1	1	.	-	.	-	1	-	1	1	1	-	1	-	1	-	.	-	.	-	1	-	-	-	-		
2104	.	-	1	1	1	.	-	.	-	1	-	1	-	1	-	1	-	2s	-	.	-	.	-	2	-	1	-	-		
2206	.	-	1	2	dead																									
2208	.	-	1	1	1	.	-	.	-	1	-	1s	-	1h	-	2h	-	1h	-	.	-	.	-	1	-	1	-	-		
2310	.	-	1	1	2h1	.	-	.	-	2s	2hs	2h	dead																	
2312	.	-	1	1	1	.	-	.	-	1	-	1	-	1	-	-	-	1	-	.	-	.	-	-	-	-	-	-		
2414	.	-	1	1	1	.	-	.	dead																					
2416	.	-	1	1	1	.	-	.	-	1	-	1	-	-	-	-	-	-	-	.	-	.	-	-	-	1	-	-		
2518	.	-	1	1	1	.	-	.	-	1	-	1	-	1	-	2h	1h	2h	1	.	-	.	-	1	-	2h	1	-		
2520	.	-	1	1	1	.	-	.	-	1	-	1	-	1	-	1	-	-	-	.	-	.	-	1	-	-	-	-		

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined h = + lower back high s = + staggering

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Table 1-5 Clinical symptoms -- **Reduced grooming**

Animal	Day of the study (day of exposure)																											
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																												
2101	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2103	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2205	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2207	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2309	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2311		-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2413		-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2415		-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2517	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2519	.	-	-	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
FEMALES																												
2102	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2104	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2206	.	-	1	1	dead																							
2208	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2310	.	-	1	1	-	.	-	.	-	-	-	-	dead															
2312	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2414	.	-	1	1	-	.	-	.	dead																			
2416	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2518	.	-	1	1	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-
2520	.	-	1	1	-	.	-	.	-	-	-		-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined

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Table 1-6 Clinical symptoms -- **Hypothermia**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2103	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2205	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2207	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2309	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2311	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2413	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2415	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2517	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2519	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
FEMALES																														
2102	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2104	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2206	.	-	1	1	dead																									
2208	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2310	.	-	1	-	1	.	-	.	-	-	-	1	dead																	
2312	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2414	.	-	1	-	1	.	-	.	dead																					
2416	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2518	.	-	1	-	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2520	.	-	1	-	1	.	-	.	-	-	-		-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined

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Table 1-7 Clinical symptoms -- **Dirt around nose**

Animal	Day of the study (day of exposure)																													
	1(1)		2(2)		3(-)		4(-)		5(3)		6(4)		7(5)		8(6)		9(7)		10(-)		11(-)		12(8)		13(9)		14(10)	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
MALES																														
2101	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2103	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2205	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2207	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2309	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2311	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2413	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2415	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2517	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2519	.	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
FEMALES																														
2102	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2104	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2206	.	-	1	1	dead																									
2208	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2310	.	-	1	1	1	.	-	.	-	-	-	1	dead																	
2312	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2414	.	-	1	1	1	.	-	.	dead																					
2416	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2518	.	-	1	1	1	.	-	.	-	-	-	-	-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-
2520	.	-	1	1	1	.	-	.	-	-	-		-	-	-	-	-	-	-	.	-	.	-	-	-	-	-	-	-	-

No findings noted for control animals

a = before exposure b = after exposure
- = no effect 1 = slight 2 = moderate to severe . = not examined

Table 2 Body weight (g)

Group ppm	Animal number	Day of study -7	-3	1	5	8	12	14
MALES								
0	1101	320	352	382	395	401	416	428
	1103	321	345	363	370	379	388	390
	1205	325	343	360	361	362	373	373
	1207	323	370	397	407	399	402	403
	1309	337	368	387	380	373	384	385
	1311	345	388	411	422	430	445	444
	1413	348	392	409	407	399	413	406
	1415	347	377	404	406	393	404	404
	1517	357	379	393	392	391	399	395
	1519	355	383	401	401	398	404	406
		---	---	---	---	---	---	---
	Mean	338	370	391	394	392	403	403
10000	2101	319	354	380	341	333	373	358
	2103	319	349	364	356	362	381	377
	2205	325	358	378	369	366	382	377
	2207	324	359	386	370	365	387	376
	2309	328	367	390	391	381	401	385
	2311	331	369	396	396	392	414	403
	2413	353	395	426	423	410	431	423
	2415	350	376	399	361	347	391	384
	2517	357	401	428	401	393	414	406
	2519	353	386	398	400	388	403	403
		---	---	---	---	---	---	---
	Mean	336	371	394	381	374	398	389
FEMALES								
0	1102	242	264	261	266	264	271	281
	1104	250	269	269	271	272	276	285
	1206	251	258	262	265	261	260	262
	1208	250	255	257	260	261	262	260
	1310	255	274	289	294	289	295	293
	1312	254	270	263	277	282	285	286
	1414	257	281	280	293	288	297	293
	1416	256	274	279	280	281	283	291
	1518	263	282	275	283	282	289	289
	1520	260	274	285	288	276	283	291
		---	---	---	---	---	---	---
	Mean	254	270	272	278	275	280	283
10000	2102	244	258	258	268	272	294	280
	2104	243	257	267	270	270	298	287
	2206	251	262	267	a			
	2208	250	260	265	258	251	278	266
	2310	253	254	252	238	a		
	2312	252	259	264	259	269	278	279
	2414	259	274	280	a			
	2416	256	279	277	274	283	293	294
	2518	259	277	277	264	252	282	261
	2520	263	281	281	280	283	290	285
		---	---	---	---	---	---	---
	Mean	253	266	269	264	269	288	279

a animal dead

Table 3 Body weight gain (g)

Group ppm	Animal number	During days -7 -3	-3 1	1 5	5 8	8 12	12 14
MALES							
0	1101	32	30	13	6	15	12
	1103	24	19	7	9	8	3
	1205	19	16	2	1	11	- 1
	1207	47	27	10	- 8	3	0
	1309	31	18	- 6	- 7	11	2
	1311	44	23	12	8	15	- 2
	1413	44	17	- 2	- 8	14	- 7
	1415	30	27	2	-13	11	- 1
	1517	22	14	- 0	- 2	8	- 4
	1519	28	18	0	- 3	6	3
		---	---	---	---	---	---
	Mean	32	21	4	- 2	10	1
10000	2101	35	27	-39	- 8	39	-15
	2103	30	15	- 8	6	19	- 4
	2205	33	20	- 9	- 3	16	- 6
	2207	35	26	-16	- 4	22	-12
	2309	39	24	1	-11	20	-16
	2311	38	27	1	- 4	22	-11
	2413	43	31	- 3	-14	21	- 8
	2415	26	23	-38	-14	44	- 7
	2517	44	27	-27	- 8	22	- 9
	2519	33	12	2	-12	15	- 0
		---	---	---	---	---	---
	Mean	36	23	-14	- 7	24	- 9
FEMALES							
0	1102	22	- 3	5	- 3	7	11
	1104	19	- 0	2	1	5	9
	1206	7	3	3	- 4	- 1	3
	1208	5	2	3	1	1	- 2
	1310	19	15	5	- 5	6	- 2
	1312	16	- 6	14	5	3	1
	1414	24	- 1	13	- 5	10	- 4
	1416	19	5	1	1	2	8
	1518	19	- 7	8	- 1	7	- 1
	1520	14	11	3	-12	8	7
		---	---	---	---	---	---
	Mean	16	2	6	- 2	5	3
10000	2102	15	0	10	4	23	-14
	2104	14	11	3	0	27	-10
	2206	11	5	a			
	2208	10	5	- 7	- 7	27	-12
	2310	1	- 3	-14	a		
	2312	7	6	- 5	10	9	1
	2414	15	6	a			
	2416	23	- 2	- 3	10	9	1
	2518	18	0	-14	-12	30	-21
	2520	18	- 1	- 1	3	7	- 5
		---	---	---	---	---	---
	Mean	13	3	- 4	1	19	- 9

a animal dead

Table 4 Food consumption (g)

Group ppm	Animal number	During days -7 -3	-3 1	1 5	5 8	8 12	12 14
MALES							
0	1101	118	92	113	71	111	54
	1103	130	92	109	76	117	51
	1205	107	76	98	65	95	44
	1207	136	108	124	67	98	47
	1309	121	86	94	59	101	47
	1311	135	101	121	85	112	55
	1413	133	101	107	66	106	47
	1415	127	99	110	66	103	47
	1517	118	90	108	71	104	51
	1519	118	87	115	74	104	52
		---	---	---	---	---	---
	Mean	124	93	110	70	105	49
10000	2101	123	91	54	53	112	40
	2103	103	79	70	62	98	44
	2205	129	96	96	61	111	47
	2207	133	102	77	57	103	42
	2309	120	93	97	62	96	42
	2311	129	103	95	70	107	47
	2413	149	111	107	62	115	46
	2415	116	96	32	38	109	48
	2517	151	122	86	69	119	51
	2519	135	96	96	63	98	44
		---	---	---	---	---	---
	Mean	129	99	81	60	107	45
FEMALES							
0	1102	92	64	85	56	86	43
	1104	90	60	75	56	80	39
	1206	92	62	96	53	75	43
	1208	84	60	77	47	70	35
	1310	93	73	82	52	76	42
	1312	98	69	89	62	84	40
	1414	110	68	95	65	103j	51
	1416	102	67	101	64	89	44
	1518	109	77	86	53	88	42
	1520	107	71	96	51	84	46
		---	---	---	---	---	---
	Mean	98	67	88	56	81	42
10000	2102	90	62	80	63	110j	42
	2104	93	65	71	46	84	39
	2206	89	60	a			
	2208	81	64	37	30	81	36
	2310	75	59	11	a		
	2312	94	77	57	52	89	37
	2414	100	70	a			
	2416	100	62	76	69	132j	35
	2518	109	61	55	38	102j	38
	2520	94	70	61	46	75	32
		---	---	---	---	---	---
	Mean	92	65	56	49	82	37

a animal dead
j unreliable value; excluded from mean

Table 6-1 Haematology

MALES

Group ppm	Animal number	RBC 10^{12}/l	HGB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLT 10^{9}/l
0	1101	8.09	9.9	0.440	54.4	1.224	22.5	975
	1103	8.23	10.4	0.459	55.8	1.264	22.7	1071
	1205	8.86	10.5	0.471	53.2	1.185	22.3	1136
	1207	9.30	11.2	0.503	54.1	1.204	22.3	1150
	1309	8.43	10.1	0.446	52.9	1.198	22.6	993
	1311	8.35	10.1	0.455	54.5	1.210	22.2	1150
	1413	8.84	11.0	0.489	55.3	1.244	22.5	998
	1415	9.35	10.9	0.483	51.7	1.166	22.6	1000
	1517	7.93	10.6	0.433	54.6	1.337	24.5	971
	1519	8.83	10.7	0.476	53.9	1.212	22.5	1055
	Mean	8.62	10.5	0.466	54.0	1.224	22.7	1050
	Stdev	0.49	0.4	0.023	1.2	0.048	0.7	73
10000	2101 x)	9.15	11.3	0.501	54.8	1.235	22.6	779
	2103 x)	8.00	10.3	0.461	57.6	1.288	22.3	1115
	2205	8.68	10.9	0.479	55.2	1.256	22.8	937
	2207	8.51	10.8	0.476	55.9	1.269	22.7	900
	2309 x)	8.09	10.2	0.450	55.6	1.261	22.7	1075
	2311 x)	8.13	10.5	0.467	57.4	1.292	22.5	1026
	2413	8.09	11.2	0.491	60.7	1.384	22.8	961
	2415 x)	7.33	9.2	0.431	58.8	1.255	21.3	1696
	2517 x)	8.73	11.4	0.498	57.0	1.306	22.9	908
	2519	7.77	10.1	0.430	55.3	1.300	23.5	962
	Mean	8.25	10.6	0.468	56.8	1.285	22.6	1036
	Stdev	0.53	0.7	0.026	1.9	0.042	0.6	251

x) Normoblasts present, 2101, 2103, 2309 and 2517 n=1, 2415 n=4, 2311 n=5

Table 5 Water consumption (g)

Group ppm	Animal number	During days -7 to -3	-3 to 1	1 to 5	5 to 8	8 to 12	12 to 14
MALES							
0	1101	150	94	127	87	121	63
	1103	229	105	230	125	204	92
	1205	123	99	138	93	156	63
	1207	204	158	143	103	147	72
	1309	145	112	143	116	184	84
	1311	158	124	173	127	154	85
	1413	251	182	209	130	186	91
	1415	290	272	276	169	228	112
	1517	107	76	97	77	109	56
	1519	162	145	200	126	150	91
		---	---	---	---	---	---
	Mean	182	137	173	115	164	81
10000	2101	138	120	230	268	255	102
	2103	103	85	107	98	122	58
	2205	125	100	117	87	133	58
	2207	154	135	160	137	192	80
	2309	148	249	208	96	141	65
	2311	169	113	133	123	153	75
	2413	253	245	265	202	281	106
	2415	143	115	72	102	171	79
	2517	221	166	182	142	199	93
	2519	189	117	141	90	123	69
		---	---	---	---	---	---
	Mean	164	144	161	134	177	79
FEMALES							
0	1102	117	91	120	84	144	73
	1104	124	136	114	91	127	66
	1206	94	58	87	60	82	51
	1208	139	96	166	95	162	84
	1310	110	82	111	73	113	56
	1312	119	83	189	97	136	56
	1414	162	97	157	99	171	78
	1416	133	81	138	100	143	78
	1518	149	94	147	95	134	74
	1520	149	88	164	132	149	80
		---	---	---	---	---	---
	Mean	130	90	139	93	136	69
10000	2102	138	85	190	221	250	121
	2104	98	63	126	140	210	109
	2206	115	74	a			
	2208	97	103	72	56	161	98
	2310	114	77	40	a		
	2312	120	89	236	193	233	96
	2414	134	103	a			
	2416	153	103	217	170	213	84
	2518	127	66	133	185	273	152
	2520	99	62	95	87	112	63
		---	---	---	---	---	---
	Mean	119	83	139	150	207	103

a animal dead

Table 6-2 Haematology

FEMALES

Group ppm	Animal number	RBC 10^{12}/l	HGB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLT 10^9/l
0	1102	7.40	9.7	0.426	57.6	1.311	22.8	1049
	1104	7.58	9.8	0.424	55.9	1.293	23.1	934
	1206	7.97	10.1	0.449	56.3	1.267	22.5	843
	1208	7.72	9.9	0.425	55.1	1.282	23.3	991
	1310	7.40	10.2	0.429	58.0	1.378	23.8	1041
	1312	7.86	10.2	0.449	57.1	1.298	22.7	950
	1414	7.78	10.2	0.435	55.9	1.311	23.4	910
	1416	7.98	9.9	0.430	53.9	1.241	23.0	985
	1518	8.29	10.7	0.448	54.0	1.291	23.9	969
	1520 x)	7.66	10.1	0.440	57.4	1.319	23.0	1036
	Mean	7.76	10.1	0.436	56.1	1.299	23.2	971
	Stdev	0.28	0.3	0.010	1.4	0.036	0.5	65
10000	2102	7.64	10.2	0.441	57.7	1.335	23.1	1319
	2104 x)	7.57	10.0	0.444	58.7	1.321	22.5	1139
	2206 a)							
	2208zx)	6.48	8.7	0.402	62.0	1.343	21.6	1273
	2310 a)							
	2312 x)	7.37	9.6	0.421	57.1	1.303	22.8	985
	2414 a)							
	2416 x)	7.47	9.8	0.428	57.3	1.312	22.9	905
	2518zx)	8.05	10.5	0.465	57.8	1.304	22.6	1232
	2520 x)	8.06	10.3	0.450	55.8	1.278	22.9	1050
	Mean	7.5	9.9	0.4	58.1	1.3	22.6	1129
	Stdev	0.5	0.6	0.0	1.9	0.0	0.5	155

a) Animal dead
x) Normoblasts present, 1520 n=1, 2104 n=6, 2208 n=23, 2312 n=3, 2416 n=1, 2518 n=5, 2520 n=2,
z) Howell Jolly bodies present, 2208 n=85, 2518 n=32

Table 6-3 Haematology

MALES

Group ppm	Animal number	WBC 10^9/l	Neut. %	Lyph. %	Mono. %	Eos. %	Neut. 10^9/l	Lyph. 10^9/l	Mono. 10^9/l	Eos. 10^9/l
0	1101	17.7	28	70	2	0	4.96	12.39	0.35	0.00
	1103	17.0	10	90	0	0	1.70	15.30	0.00	0.00
	1205	20.8	8	90	0	2	1.66	18.72	0.00	0.42
	1207	13.5	12	86	2	0	1.62	11.61	0.27	0.00
	1309	18.5	15	84	0	1	2.78	15.54	0.00	0.19
	1311	21.2	23	72	4	1	4.88	15.26	0.85	0.21
	1413	12.3	28	71	1	0	3.44	8.73	0.12	0.00
	1415	11.5	6	91	3	0	0.69	10.47	0.35	0.00
	1517	12.4	7	89	3	1	0.87	11.04	0.37	0.12
	1519	12.3	10	86	1	3	1.23	10.58	0.12	0.37
	Mean	15.7	15	83	2	1	2.38	12.96	0.24	0.13
	Stdev	3.7	9	9	1	1	1.57	3.10	0.26	0.16
10000	2101	4.6	31	68	0	1	1.43	3.13	0.00	0.05
	2103	8.7	27	67	3	3	2.35	5.83	0.26	0.26
	2205	7.5	23	69	5	3	1.73	5.18	0.38	0.23
	2207	7.7	24	74	2	0	1.85	5.70	0.15	0.00
	2309	7.9	24	74	1	1	1.90	5.85	0.08	0.08
	2311	5.9	30	67	0	3	1.77	3.95	0.00	0.18
	2413	9.2	28	67	3	2	2.58	6.16	0.28	0.18
	2415	6.0	22	75	1	2	1.32	4.50	0.06	0.12
	2517	5.7	33	63	1	3	1.88	3.59	0.06	0.17
	2519	8.9	23	76	0	1	2.05	6.76	0.00	0.09
	Mean	7.2	27	70	2	2	1.88	5.06	0.13	0.14
	Stdev	1.6	4	4	2	1	0.38	1.21	0.13	0.08

Table 6-4 Haematology

FEMALES

Group ppm	Animal number	WBC $10^9/l$	Neut. %	Lyph. %	Mono. %	Eos. %	Neut. $10^9/l$	Lyph. $10^9/l$	Mono. $10^9/l$	Eos. $10^9/l$
0	1102	4.9	13	86	1	0	0.64	4.21	0.05	0.00
	1104	8.7	14	84	1	1	1.22	7.31	0.09	0.09
	1206	5.4	32	67	1	0	1.73	3.62	0.05	0.00
	1208	7.4	29	67	1	3	2.15	4.96	0.07	0.22
	1310	11.6	16	81	3	0	1.86	9.40	0.35	0.00
	1312	5.7	20	78	2	0	1.14	4.45	0.11	0.00
	1414	6.6	21	79	0	0	1.39	5.21	0.00	0.00
	1416	8.4	15	83	1	1	1.26	6.97	0.08	0.08
	1518	5.9	10	86	2	2	0.59	5.07	0.12	0.12
	1520	6.9	11	88	0	1	0.76	6.07	0.00	0.07
	Mean	7.2	18	80	1	1	1.27	5.73	0.09	0.06
	Stdev	2.0	7	7	1	1	0.52	1.74	0.10	0.07
10000	2102	7.1	11	88	0	1	0.78	6.25	0.00	0.07
	2104	3.6	25	68	4	3	0.90	2.45	0.14	0.11
	2206 a)									
	2208	4.6	19	76	2	3	0.87	3.50	0.09	0.14
	2310 a)									
	2312	10.4	38	61	0	1	3.95	6.34	0.00	0.10
	2414 a)									
	2416	4.9	17	81	0	2	0.83	3.97	0.00	0.10
	2518	5.5	8	86	2	4	0.44	4.73	0.11	0.22
	2520	4.1	19	73	1	7	0.78	2.99	0.04	0.29
	Mean	5.7	20	76	1	3	1.22	4.3	0.06	0.15
	Stdev	2.3	10	10	1	2	1.21	1.5	0.06	0.08

a) Animal dead.

Table 7-1 Clinical chemistry

MALES

Group ppm	Animal number	AST U/L	ALT U/L	ALP U/L	Gluc mmol/l	Album g/l	Urea mmol/l
0	1101	46	24	291	5.31	43.2	5.76
	1103 e)						
	1205.	85	31	333	6.07	43.5	4.15
	1207	55	24	394	7.16	45.4	4.72
	1309	81	32	301	4.85	44.9	4.16
	1311	68	38	276	4.85	38.5h)	4.56
	1413	68	33	280	5.61	45.0	4.98
	1415	69	26	360	5.78	47.2	4.45
	1517	75	30	186	5.66	44.3	4.35
	1519	57	24	273	6.31	42.3	5.57
		---	---	---	-----	----	-----
	Mean	67	29	299	5.73	43.8	4.74
10000	2101	66	27	208	6.18	48.3	4.66
	2103	75	36	228	7.79	45.0	4.68
	2205	76	31	195	6.08	43.8	4.76
	2207	48	19	233	7.09	47.1	4.53
	2309	55	22	267	5.10	43.9	4.11
	2311	86	26	249	5.28	42.7	3.95
	2413	471 h)	70	219	7.85	37.7h)	b)
	2415	61	23	291	6.33	38.0h)	6.86
	2517	55	22	189	6.43	44.1	5.56
	2519	93	23	230	7.82	43.0	5.00
		---	---	---	-----	----	-----
	Mean	109	30	231	6.60	43.4	4.90

b) Missing value
e) Unsuitable sample
h) Result confirmed

Table 7-2 Clinical chemistry

FEMALES

Group ppm	Animal number	AST U/L	ALT U/L	ALP U/L	Gluc mmol/l	Album g/l	Urea mmol/l
0	1102	52	27	150	6.84	50.0	5.33
	1104	73	28	183	5.50	47.0	5.36
	1206	61	24	121	8.28	48.4	6.26
	1208	57	24	119	7.29	53.2	7.16
	1310	55	23	148	7.48	47.2	5.83
	1312	52	24	78	6.96	49.5	6.80
	1414	71	23	155	8.25	46.7	4.50
	1416	16	32	140	7.68	46.8	5.56
	1518	59	31	124	7.47	51.8	4.23
	1520	47	22	169	8.39	48.5	5.75
		---	---	---	-----	----	-----
	Mean	54	26	138	7.41	48.9	5.68
10000	2102	73	24	173	5.24	47.5	5.85
	2104	124	25	177	6.26	47.7	5.48
	2206 a)						
	2208	73	22	141	7.40	48.9	4.92
	2310 a)						
	2312	66	24	208	9.60	50.4	4.87
	2414 a)						
	2416	55	21	150	6.06	43.9	4.44
	2518	50	19	170	7.62	44.2	4.42
	2520	62	27	195	7.47	48.4	4.55
		---	---	---	-----	----	-----
	Mean	72	23	173	7.09	47.3	4.93

a) Animal dead

Table 7-3 Clinical chemistry

MALES

Group ppm	Animal number	Creat µmol/l	Chol mmol/l	Trig mmol/l	Oestra-diol ng/l	Testo-steron ng/l
0	1101	38	1.07	0.51	0.57	1760
	1103 e)					
	1205	38	1.08	0.46	0.42	1211
	1207	40	1.19	0.82	0.31	1444
	1309	39	1.03	0.45	0.70	2248
	1311	39	1.12	0.46	0.40	614
	1413	46	1.34	0.64	0.95	1500
	1415	37	.94	0.51	0.93	3122
	1517	39	.92	0.52	0.57	1502
	1519	48	.98	0.37	0.75	2869
		---	----	----	---	----
	Mean	40	1.07	0.53	0.62	1808
10000	2101	49	1.59	0.31	0.67	1089
	2103	52	1.14	0.61	0.44	975
	2205	42	1.90	0.50	0.46	756
	2207	38	2.11	0.71	0.61	1801
	2309	46	1.09	0.47	0.51	2296
	2311	40	1.21	0.81	0.40	2314
	2413	b)	1.72	0.48	0.93	1252
	2415	48	1.41	0.32	0.28 q)	587
	2517	43	2.05	0.39	0.70	3321
	2519	52	1.55	0.65	0.42	1134
		---	----	----	---	----
	Mean	46	1.58	0.52	0.54	1553

b) Missing value
e) Unsuitable sample
q) Value below limit of quantification (included in means and statistics)

Table 7-4 Clinical chemistry

FEMALES

Group ppm	Animal number	Creat µmol/l	Chol mmol/l	Trig mmol/l	Oestra-diol ng/l	Testo-steron ng/l	
0	1102	43	1.48	0.32	31.64	226	
	1104•	57	1.33	0.41	35.17	256	
	1206	61	1.56	0.20	0.91	44	q)
	1208	57	1.71	0.50	0.36	38	q)
	1310	54	2.01	0.38	0.36	82	
	1312	46	1.95	0.52	21.33	181	
	1414	56	3.20	0.53	1.37	70	
	1416	52	1.96	0.51	39.09	189	
	1518	47	2.75	0.66	10.74	43	q)
	1520	47	1.45	0.26	26.62	85	
		---	----	----	-----	---	
	Mean	52	1.94	0.43	16.76	121	
10000	2102	48	1.42	0.39	3.19	74	
	2104	48	1.88	0.66	6.99	179	
	2206 a)						
	2208	42	1.53	0.72	1.93	110	
	2310 a)						
	2312	61	2.14	0.53	10.82	126	
	2414 a)						
	2416	55	1.39	0.41	4.27	96	
	2518	46	1.88	0.37	4.74	154	
	2520	51	1.69	0.47	16.08	56	q)
		---	----	----	-----	---	
	Mean	50	1.70	0.51	6.86	114	

a) Animal dead
q) Value below limit of quantification (included in means and statistics)

Table 7-5 Clinical chemistry

MALES

Group ppm	Animal number	Ca++ mmol/l	In.pho mmol/l	Na+ mmol/l	K+ mmol/l
0	1101	2.56	2.73	146	3.49
	1103 e)				
	1205	2.58	2.38	146	4.33
	1207	2.65	2.32	149	3.49
	1309	2.53	2.42	148	3.37
	1311	2.62	2.37	147	3.50
	1413	2.61	2.60	147	3.48
	1415	2.62	2.64	147	4.02
	1517	2.61	2.35	145	4.28
	1519	2.59	2.41	146	3.74
		----	----	---	----
	Mean	2.60	2.47	147	3.74
10000	2101	2.77	2.39	145	3.47
	2103	2.48	2.34	142	3.21
	2205	2.45	2.46	b)	b)
	2207	2.63	2.44	147	3.42
	2309	2.72	2.22	145	3.60
	2311	2.59	2.41	147	3.51
	2413	b)	3.58 h)	b)	b)
	2415	2.57	2.76	148	3.63
	2517	2.72	2.40	145	3.43
	2519	2.60	2.20	145	3.45
		----	----	---	----
	Mean	2.61	2.52	145	3.47

b) Missing value
e) Unsuitable sample
h) Result confirmed.

Table 7-6 Clinical chemistry

FEMALES

Group ppm	Animal number	Ca++ mmol/l	In.pho mmol/l	Na+ mmol/l	K+ mmol/l
0	1102	2.60	1.91	147	3.76
	1104	2.63	1.94	147	4.39
	1206	2.53	1.65	146	3.09
	1208	2.61	1.49	145	3.11
	1310	2.61	2.13	146	3.74
	1312	2.67	1.84	146	3.66
	1414	2.65	1.57	146	3.41
	1416	2.67	2.15	144	3.58
	1518	2.69	1.77	145	3.69
	1520	2.54	1.66	143	4.00
		----	----	---	----
	Mean	2.62	1.81	145	3.64
10000	2102	2.76	2.94	144	3.84
	2104	2.66	2.16	140	3.65
	2206 a)				
	2208	2.69	2.54	145	3.97
	2310 a)				
	2312	2.45	1.82	142	3.14
	2414 a)				
	2416	2.58	2.11	147	3.47
	2518	2.63	2.31	146	4.00
	2520	2.61	2.21	144	3.54
		----	----	---	----
	Mean	2.63	2.30	144	3.66

a) Animal dead

Table 8-1 Urine analysis

MALES

Group ppm	Animal number	Vol ml	S.G.	Osmo osm/kg	Na mmol/l	K mmol/l	Na mmol	K mmol	F mg/l	F µmol
0	1101	9.7	1.031	1.072	62.9	133.9	0.61	1.30	2.25	22
	1103	11.4	1.022	0.709	16.5	84.9	0.19	0.97	1.20	14
	1205	39.5	1.011	0.372	18.9	37.2	0.75	1.47	0.85	34
	1207	21.3	1.013	0.413	4.4	37.7	0.09	0.80	0.70	15
	1309	26.0	1.017	0.579	20.3	67.5	0.53	1.76	1.30	34
	1311	29.5	1.018	0.603	29.7	65.3	0.88	1.93	1.15	34
	1413	44.0	1.008	0.262	12.8	23.4	0.56	1.03	0.70	31
	1415	23.6	1.015	0.487	28.3	44.9	0.67	1.06	1.05	25
	1517	22.2	1.016	0.501	22.3	46.4	0.50	1.03	1.00	22
	1519	17.5	1.025	0.854	32.3	83.4	0.57	1.46	1.55	27
	Mean	24.5	1.018	0.585	24.8	62.5	0.53	1.28	1.18	25.8
	Stdev	11.0	0.007	0.241	15.8	32.4	0.24	0.37	0.46	7.6
10000	2101	44.0	1.007	0.235	10.8	16.8	0.48	0.74	1.15	51
	2103	38.5	1.008	0.424	4.8	24.3	0.18	0.94	1.30	50
	2205	13.0	1.018	0.606	19.3	55.5	0.25	0.72	3.90	51
	2207	14.0	1.023	0.795	17.1	80.2	0.24	1.12	5.80	95
	2309	33.8	1.010	0.313	4.1	27.0	0.14	0.91	2.10	71
	2311	28.5	1.011	0.366	7.7	37.1	0.22	1.06	3.20	91
	2413	29.5	1.010	0.321	14.1	22.5	0.42	0.66	1.55	46
	2415	28.0	1.012	0.411	23.3	46.2	0.65	1.29	2.25	63
	2517	36.0	1.009	0.300	6.4	24.9	0.23	0.90	2.60	72
	2519	11.5	1.022	0.713	14.7	74.6	0.17	0.86	4.80	55
	Mean	27.7	1.013	0.448	12.2	40.9	0.30	0.92	2.91	64.5
	Stdev	11.3	0.006	0.190	6.5	22.6	0.16	0.19	1.60	17.4

Table 8-2 Urine analysis

FEMALES

Group ppm	Animal number	Vol ml	S.G.	Osmo osm/kg	Na mmol/l	K mmol/l	Na mmol	K mmol	F mg/l	F µmol
0	1102	26.0	1.009	0.286	5.9	30.4	0.15	0.79	0.85	22
	1104	21.2	1.012	0.368	5.8	39.7	0.12	0.84	0.95	20
	1206	3.5	1.043	1.649	49.7	165.7	0.17	0.58	2.40	8
	1208	5.9	1.028	1.010	28.7	116.4	0.17	0.69	1.75	10
	1310	14.4	1.014	0.463	15.7	38.5	0.23	0.55	0.95	14
	1312	11.0	1.017	0.562	13.7	50.5	0.15	0.56	1.05	12
	1414	5.7	1.030	1.060	66.0	110.6	0.38	0.63	1.70	10
	1416	13.7	1.016	0.546	6.7	55.6	0.09	0.76	1.10	15
	1518	20.4	1.012	0.387	5.8	32.2	0.12	0.66	0.75	15
	1520	10.3	1.019	0.635	8.7	58.2	0.09	0.60	1.25	13
	Mean	13.2	1.020	0.697	20.7	69.8	0.17	0.67	1.28	13.9
	Stdev	7.4	0.011	0.423	21.2	45.5	0.08	0.10	0.52	4.4
10000	2102	36.0	1.007	0.244	2.0	17.7	0.07	0.64	1.25	45
	2104	51.0	1.005	0.183	15.1	18.6	0.77	0.95	1.20	61
	2206 a)									
	2208	48.5	1.005	0.182	5.8	16.7	0.28	0.81	1.10	53
	2310 a)									
	2312	22.8	1.012	0.398	12.6	42.7	0.29	0.97	3.45	79
	2414 a)									
	2416	13.5	1.017	0.561	7.5	43.8	0.10	0.59	2.50	34
	2518	30.0	1.007	0.252	13.3	23.1	0.40	0.69	1.55	47
	2520	7.7	1.025	0.859	40.2	89.7	0.31	0.69	3.35	26
	Mean	29.9	1.011	0.383	13.8	36.0	0.32	0.76	2.06	49.3
	Stdev	16.5	0.007	0.250	12.6	26.4	0.23	0.15	1.03	17.5

a) Animal dead.

Table 8-3 Urine analysis

MALES

Group ppm	Animal number	ALP U/l	ALP U/mmol Creat	ALP U/g Protein	GGT U/l	GGT U/mmol Creat	GGT U/g Protein
0	1101	235.9	23.4	327	1052	104	1459
	1103	35.5	4.8	134	752	100	2837
	1205	10.7	3.1	113	390	111	4106
	1207	76.3	17.5	515	500	115	3378
	1309	61.1	11.2	359	836	154	4917
	1311	63.4	12.7	576	1153	232	10483
	1413	8.6	3.2	122	287	108	4047
	1415	78.4	16.8	436	621	133	3452
	1517	35.3	7.0	275	468	94	3658
	1519	138.1	17.2	822	804	100	4787
	Mean	74.3	11.7	368	686	125	4313
	Stdev	68.2	7.0	228	284	41	2384
10000	2101	4.3	2.3	70	259	139	4175
	2103	4.7	1.8	81	286	107	4934
	2205	27.4	4.6	175	1306	218	8321
	2207	195.4	29.8	1073	1704	260	9362
	2309	11.4	3.7	134	334	109	3929
	2311	10.7	2.8	132	460	122	5681
	2413	8.8	2.8	116	711	230	9356
	2415	9.3	2.8	124	223	67	2967
	2517	8.3	2.8	163	357	121	6993
	2519	18.3	2.4	120	1139	150	7494
	Mean	29.9	5.6	219	678	152	6321
	Stdev	58.5	8.6	302	524	63	2314

Table 8-4 Urine analysis

FEMALES

Group ppm	Animal number	ALP U/l	ALP U/mmol Creat	ALP U/g Protein	GGT U/l	GGT U/mmol Creat	GGT U/g Protein
0	1102	19.6	7.7	258	78	31	1026
	1104	28.2	8.2	307	135	39	1472
	1206	203.5	14.0	672	1007	69	3323
	1208	79.6	8.1	384	382	39	1845
	1310	8.2	1.8	79	356	78	3419
	1312	3.4	0.8	25	169	37	1261
	1414	113.0	12.2	568	554	60	2784
	1416	11.3	2.4	126	156	33	1734
	1518	9.7	2.7	111	196	54	2253
	1520	50.6	8.8	294	188	33	1095
	Mean	52.7	6.7	282	322	47	2021
	Stdev	63.9	4.6	212	280	17	890
10000	2102	4.0	2.0	51	156	79	1978
	2104	6.8	4.6	116	49	33	836
	2206 a)						
	2208	4.7	2.8	92	27	16	528
	2310 a)						
	2312	4.0	1.1	59	170	49	2541
	2414 a)						
	2416	19.2	3.6	196	399	75	4074
	2518	2.4	1.0	55	36	16	828
	2520	34.6	4.0	298	592	68	5105
	Mean	10.8	2.7	124	204	48	2270
	Stdev	11.9	1.4	92	214	27	1762

a) Animal dead

Table 8-5 Urine analysis

MALES

Group ppm	Animal number	Creat µmol/l	Creat µmol	Protein mg/l	Prot mg	NAG U/l	NAG U/mmol Creat	NAG U/g Protein
0	1101	10091	98	721	6.99	10.7	1.1	15
	1103	7481	85	265	3.02	10.7	1.4	40
	1205	3501	138	95	3.75	7.6	2.2	80
	1207	4349	93	148	3.15	9.9	2.3	67
	1309	5431	141	170	4.42	9.2	1.7	54
	1311	4979	147	110	3.25	7.5	1.5	69
	1413	2663	117	71	3.12	5.0	1.9	71
	1415	4677	110	180	4.25	8.5	1.8	47
	1517	5004	111	128	2.84	7.1	1.4	55
	1519	8036	141	168	2.94	9.6	1.2	57
	Mean	5621	118	206	3.77	8.6	1.7	56
	Stdev	2257	22	189	1.26	1.8	0.4	19
10000	2101	1860	82	62	2.73	4.1	2.2	65
	2103	2681	103	58	2.23	4.3	1.6	74
	2205	5979	78	157	2.04	11.7	1.9	74
	2207	6546	92	182	2.55	10.0	1.5	55
	2309	3070	104	85	2.87	4.3	1.4	50
	2311	3767	107	81	2.31	4.7	1.2	58
	2413	3092	91	76	2.24	6.5	2.1	86
	2415	3313	93	75	2.10	5.7	1.7	76
	2517	2946	106	51	1.84	4.7	1.6	92
	2519	7577	87	152	1.75	10.3	1.4	68
	Mean	4083	94	98	2.27	6.6	1.7	70
	Stdev	1908	10	47	0.36	2.9	0.3	13

Table 8-6 Urine analysis

FEMALES

Group ppm	Animal number	Creat µmol/l	Creat µmol	Protein mg/l	Prot mg	NAG U/l	NAG U/mmol Creat	NAG U/g Protein
0	1102	2545	66	76	1.98	5.6	2.2	74
	1104	3445	73	92	1.95	7.6	2.2	83
	1206	14518	51	303	1.06	22.7	1.6	75
	1208	9764	58	207	1.22	19.1	2.0	92
	1310	4565	66	104	1.50	10.2	2.2	98
	1312	4529	50	134	1.47	9.3	2.1	69
	1414	9243	53	199	1.13	18.3	2.0	92
	1416	4679	64	90	1.23	6.8	1.5	76
	1518	3637	74	87	1.77	7.9	2.2	90
	1520	5731	59	172	1.77	8.8	1.5	51
	Mean	6266	61	146	1.51	11.6	1.9	80
	Stdev	3751	9	73	0.34	6.0	0.3	14
10000	2102	1984	71	79	2.84	3.4	1.7	43
	2104	1494	76	59	3.01	2.8	1.9	47
	2206 a)							
	2208	1663	81	51	2.47	3.8	2.3	74
	2310 a)							
	2312	3465	79	67	1.53	7.9	2.3	118
	2414 a)							
	2416	5332	72	98	1.32	9.6	1.8	98
	2518	2334	70	44	1.32	4.3	1.8	98
	2520	8674	67	116	0.89	8.4	1.0	72
	Mean	3564	74	73	1.91	5.7	1.8	79
	Stdev	2619	5	26	0.84	2.8	0.4	28

a) Animal dead

Table 8-7 Urine analysis

Group ppm	Animal number	pH	Leu	Nit	Pro	Glu	Ket	Ubg	Bil	Ery
MALES										
0	1101	7	++	-	+	-	(+)	-	-	+
	1103	6	(+)	-	(+)	-	-	-	-	+
	1205	7	-	(+)	-	-	-	-	-	(+)
	1207	6	(+)	-	-	-	-	-	-	-
	1309	7	(+)	-	(+)	-	-	-	-	(+)
	1311	7	-	-	-	-	-	-	-	+++
	1413	7	-	-	-	-	-	-	-	-
	1415	7	(+)	-	-	-	-	-	-	(+)
	1517	7	(+)	-	(+)	-	-	-	-	(+)
	1519	6	(+)	-	(+)	-	-	-	-	(+)
10000	2101	6	-	-	-	-	-	-	-	(+)
	2103	6	-	-	-	-	-	-	-	-
	2205	6	(+)	-	(+)	-	-	-	-	(+)
	2207	6	(+)	-	(+)	-	-	-	-	(
	2309	6	-	-	-	-	-	-	-	(+)
	2311	6	-	-	-	-	-	-	-	(+)
	2413	6	-	-	-	-	-	-	-	-
	2415	6	-	-	-	-	-	-	-	(+)
	2517	6	-	-	-	-	-	-	-	-
	2519	6	-	-	(+)	-	-	-	-	(+)
FEMALES										
0	1102	7	-	-	-	-	-	-	-	-
	1104	6	-	-	-	-	-	-	-	-
	1206	6	(+)	-	+	-	(+)	(+)	(+)	(+)
	1208	6	(+)	-	-	-	-	-	-	-
	1310	7	-	-	-	-	-	-	-	-
	1312	6	-	-	-	-	-	-	-	-
	1414	7	(+)	-	-	-	-	-	-	-
	1416	6	-	-	-	-	-	-	-	-
	1518	6	-	-	-	-	-	-	-	-
	1520	6	(+)	-	-	-	-	-	-	(+)
10000	2102	6	-	-	-	-	-	-	-	-
	2104	6	-	-	-	-	-	-	-	-
	2206 a)									
	2208	7	-	-	-	-	-	-	-	-
	2310 a)									
	2312	5	-	-	-	-	-	-	-	-
	2414 a)									
	2416	6	-	-	-	-	-	-	-	-
	2518	6	-	-	-	-	-	-	-	-
	2520	6	-	(+)	(+)	-	-	-	-	-

a) Animal dead

Table 9-1 Organ weights (absolute)

MALES

Group ppm	Animal number	Body weight (g)	Adrenal (mg)	Kidney (g)	Liver (g)	Lung (g)	Testes (g)
0	1101	394	74	2.74	10.27	1.44	2.97
	1103	363	71	2.58	9.89	1.41	3.57
	1205	348	61	2.34	8.29	1.58	3.20
	1207	375	67	3.08	11.06	1.46	3.78
	1309	354	61	2.43	8.69	2.05	3.28
	1311	410	75	2.92	10.42	1.61	3.50
	1413	378	85	2.73	9.80	1.48	3.44
	1415	370	65	2.57	9.02	1.72	3.45
	1517	366	82	2.64	9.23	1.59	3.04
	1519	380	66	2.44	9.07	1.54	3.44
		---	---	---	---	---	---
	Mean	374	71	2.65	9.57	1.59	3.37
10000	2101	331	60	2.26	8.90	1.53	1.75
	2103	353	61	2.21	8.28	1.45	1.72
	2205	344	58	2.55	9.36	1.42	1.83
	2207	345	76	2.99	10.33	1.45	1.68
	2309	360	67	2.38	9.07	1.48	1.92
	2311	377	63	2.59	10.71	1.46	2.02
	2413	387	88	2.79	11.12	3.72	1.95
	2415	351	67	2.49	9.66	2.43	1.61
	2517	372	88	2.43	9.95	1.67	1.61
	2519	374	62	2.66	10.27	1.41	1.86
		---	---	---	---	---	---
	Mean	359	69	2.54	9.77	1.80	1.79

Table 9-2 Organ weights (relative)

MALES

Group ppm	Animal number	Body weight (g)	Adre-nal	Kidney	Liver	Lung	Testes
0	1101	394	18.8	0.70	2.61	0.366	0.75
	1103	363	19.6	0.71	2.73	0.390	0.98
	1205	348	17.5	0.67	2.38	0.454	0.92
	1207	375	17.9	0.82	2.95	0.388	1.01
	1309	354	17.2	0.69	2.45	0.580	0.93
	1311	410	18.3	0.71	2.54	0.393	0.85
	1413	378	22.5	0.72	2.59	0.391	0.91
	1415	370	17.5	0.69	2.44	0.464	0.93
	1517	366	22.4	0.72	2.52	0.433	0.83
	1519	380	17.4	0.64	2.39	0.406	0.90
		---	----	----	----	-----	----
	Mean	374	18.9	0.71	2.56	0.427	0.90
10000	2101	331	18.1	0.68	2.69	0.462	0.53
	2103	353	17.3	0.63	2.34	0.411	0.49
	2205	344	16.9	0.74	2.72	0.413	0.53
	2207	345	22.0	0.87	2.99	0.420	0.49
	2309	360	18.6	0.66	2.52	0.411	0.53
	2311	377	16.7	0.69	2.84	0.387	0.54
	2413	387	22.7	0.72	2.87	0.961	0.50
	2415	351	19.1	0.71	2.76	0.695	0.46
	2517	372	23.7	0.65	2.68	0.448	0.43
	2519	374	16.6	0.71	2.75	0.377	0.50
		---	----	----	----	-----	----
	Mean	359	19.2	0.71	2.72	0.498	0.50

Table 9-3 Organ weights (absolute)

FEMALES

Group ppm	Animal number	Body weight (g)	Adre-nal (mg)	Kidney (g)	Liver (g)	Lung (g)
0	1102	256	69	1.83	7.25	1.30
	1104	259	84	1.71	6.63	1.25
	1206	239	79	1.60	6.38	1.15
	1208	238	76	1.58	6.39	1.32
	1310	268	82	1.57	6.81	1.25
	1312	258	69	1.76	7.28	1.30
	1414	270	78	2.04	9.02	1.35
	1416	264	83	1.68	7.46	1.27
	1518	260	92	1.85	7.17	1.21
	1520	263	71	2.08	6.98	1.33
		---	---	----	-----	----
	Mean	258	78	1.77	7.14	1.27
10000	2102	263	107	1.78	7.61	1.32
	2104	264	101	1.85	9.50	1.71
	2208	247	71	1.55	7.72	1.75
	2312	264	79	1.65	7.09	1.33
	2416	269	111	1.73	7.15	1.31
	2518	246	78	1.72	6.66	1.81
	2520	263	101	1.66	5.71	1.32
		---	---	----	-----	----
	Mean	259	93	1.71	7.35	1.51

Table 9-4 Organ weights (relative)

FEMALES

Group ppm	Animal number	Body weight (g)	Adre-nal	Kidney	Liver	Lung
0	1102	256	26.9	0.71	2.83	0.507
	1104	259	32.4	0.66	2.55	0.481
	1206	239	33.0	0.67	2.67	0.481
	1208	238	32.0	0.66	2.69	1.554
	1310	268	30.6	0.58	2.54	1.464
	1312	258	26.8	0.68	2.83	1.505
	1414	270	28.9	0.76	3.34	1.499
	1416	264	31.4	0.64	2.83	1.480
	1518	260	35.3	0.71	2.75	1.465
	1520	263	27.0	0.79	2.65	0.504
		---	----	----	----	-----
	Mean	258	30.4	0.69	2.77	0.494
10000	2102	263	40.6	0.68	2.89	0.502
	2104	264	38.3	0.70	3.60	0.648
	2208	247	28.8	0.63	3.13	1.710
	2312	264	29.9	0.62	2.68	1.503
	2416	269	41.3	0.64	2.66	1.488
	2518	246	31.7	0.70	2.71	1.735
	2520	263	38.4	0.63	2.17	0.502
		---	----	----	----	-----
	Mean	259	35.6	0.66	2.84	0.584

Table 10-1 **Listing of macroscopic observations** - Males

		Dose group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Kidney	pelvis slightly dilated	1311	2413 2517
Lung	a few red spots	1101 1517	2415
Skin	dark spots	-	2413
	superficial lesions	1311	-
Thymus	maculate	1309 1519	-

Table 10-2 **Listing of macroscopic observations** - Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Abdominal cavity	clear red fluid	-	2310	-
Eye	diffuse white area	-	2414	-
Genital area	fur stained	-	2206 2310	-
Heart	atrium dilated with blood	-	2310	-
Liver	rough surface	-	2206 2310	-
	white areas	-	2206	-
	white spot(s)	-	2414	-
	lobular pattern pronounced	-	2206	-
Lung	a few red spots	-	-	2208
	diffuse red	-	2310 2414	-
Nose	clear red fluid	-	2310	-
Skin	crust	-	2206 2414	-
Thoracic cavity	clear red fluid	-	2310	-
	contains clear fluid	-	2206 2414	-
Trachea	clear red fluid	-	2310	-
	contents foamy	-	2206 2414	-
Thymus	maculate	-	2310	2520
	red spots	-	2206 2414	-

Table 11-1 **Listing of microscopic observations** - Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Adrenal	ectopic chromaffin tissue	1205	2103
	zona fasciculata, foci of cellular alteration		
	- slight	-	2205
	- moderate	1207	2207
		1413	
		1517	
	- marked	1103	2309
		1205	2413
		1415	
	cortex areas of cellular alteration		
	- moderate	-	2519
	- marked	1101	2101
		1309	2311
		1311	2415
		1519	2517
Kidney	pyelitis	-	2207
	areas of cortical tubular necrosis	-	2205
	areas of interstitial nephritis	-	2415
	cortico-medullary junction		
	- cyst	1415	-
	- casts	-	2103
	- tubules dilated	-	2103
	cortical scar	1415	-
	localized area of basophilic cortical tubules	1413	-
		1415	
	subcapsular cyst	1413	-
	slight unilateral hydronephrosis	1311	2517
	slight bilateral hydronephrosis	-	2413
Liver	small foci of mononuclear cells		
	- a few	-	2309
	- several	1311	-

Table 11-1 **Listing of microscopic observations** - Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Lung	areas of congestion	1309 1517	2207 2415
	congested	-	2413
	localized area of fibrosis	-	2101 2103 2205 2207 2517
	areas of fibrosis	-	2311
	fibrosis	-	2413 2415
	alveolar wall slightly thickened	1415	-
	slight proliferation of lymphoid tissue	1101	-
	localized area of emphysema	1207	-
	areas of emphysema	-	2311

Table 11-1 **Listing of microscopic observations** - Males

		Dose Group (ppm)	
		0	10000
		Terminal	Terminal
Numbers Examined		10	10
Organ	Observations		
Testis	sperm cells reduced		
	- marked	-	2311
	- severe	-	2309
	sperm cells not present	-	2101 2103 2205 2207 2413 2415 2517 2519
	spermatids degenerated	-	2101 2103 2205 2207 2309 2311 2413 2415 2517 2519
	atrophy of tubules	-	2101 2103 2205 2207 2309 2311 2413 2415 2517 2519
	giant cells in tubules	-	2101 2103 2205 2415 2517 2519
Trachea	slight inflammation in submucosa	1415	-

Table 11-2 **Listing of microscopic observations** - Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Adrenal	zona fasciculata, foci of cellular alteration			
	- slight	1104	-	-
	- moderate	-	2414	2102 2416 2518
	- marked	1208 1310 1414 1520	2206	-
	cortex areas of marked cellular alteration	1102 1206 1312 1416 1518	-	2104 2208 2312 2520
Kidney	slight dilatation of convoluted tubules	-	2206	-
Liver	areas of congestion	-	2206	-
	a few small foci of mononuclear cells	1518	-	-
	areas of necrosis	-	2206 2414	-
	centrilobular parenchyma cells contain small vacuoles	-	2206 2414	-
	necrosis	-	2310	-
	swollen	-	2206	-

Table 11-2 **Listing of microscopic observations** - Females

		Dose Group (ppm)		
		0	10000	10000
		Terminal	Decedent	Terminal
Numbers Examined		10	3	7
Organ	Observations			
Lung	areas of congestion	1312	2310 2414	2518
	alveolar wall thickened			
	- slight	1520	-	-
	- moderate	-	2414	2208
	vessel oedema			
	- marginal	-	-	2312
	- moderate	-	2414	-
	- marked	-	2206	-
	- severe	-	2310	-
	localized area fibrosis	1310 1312 1416	2206 2414	2104
Trachea	slight inflammation of submucosa	-	-	2208

INDIVIDUAL MACROSCOPY AND MICROSCOPY

All observations for which an abnormality is detected are listed.
No abnormality is detected for all observed organs which are not listed.

Missing tissues	:	None
Tissues additional to protocol	:	None

Male dose-group 0 ppm

Microscopic observed organs : Adrenal Kidney Liver Lung Testis Trachea

Animal 1101 (killed on schedule)
==========

Macroscopy:	
Lung	red spot on left lung
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	slight proliferation of lymphoid tissue

Animal 1103 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration

Animal 1205 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	ectopic chromaffin tissue zona fasciculata, foci of marked cellular alteration

Animal 1207 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Lung	a single small area at the tip of the lung, distal to a constriction, shows emphysema

Animal 1309 (killed on schedule)
==========

Macroscopy:	
Thymus	maculate
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	areas of congestion

Animal 1311 (killed on schedule)
==========

Macroscopy:	
Kidney	pelvis slightly dilated - unilateral
Skin	superficial lesions on neck under left ear
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Kidney	slight unilateral hydronephrosis
Liver	several small foci of mononuclear cells

Animal 1413 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Kidney	localized area of basophilic cortical tubules
	small subcapsular cyst lined with squamous epithelium

Animal 1415 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration
Kidney	A multilocular cyst at the cortico-medullary junction, joined to the periphery by a line of basophilic tubules with associated inflammatory cells
	cortex, scar
Lung	alveolar wall sightly thickened
Trachea	slight inflammation in submucosa

Animal 1517 (killed on schedule)
==========

Macroscopy:	
Lung	a few red spots on all lung lobes
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Lung	areas of congestion

Animal 1519 (killed on schedule)
==========

Macroscopy:	
Thymus	maculate
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex

Male dose-group 10000 ppm

Microscopic observed organs : Adrenal Kidney Liver Lung Testis Trachea

Animal 2101 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	localized area of fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Animal 2103 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	ectopic chromaffin tissue
Kidney	tubules of cortico-medullary junction dilated with casts
Lung	localized area of fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Animal 2205 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of slight cellular alteration
Kidney	areas of cortical tubular necrosis
Lung	localized area of fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Animal 2207 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Kidney	pyelitis
Lung	areas of congestion
	localized area of fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules

Animal 2309 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration
Liver	a few small foci of mononuclear cells
Testis	sperm cells severely reduced
	spermatids degenerated
	atrophy of tubules

Animal 2311 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	areas of emphysema
	areas of fibrosis
Testis	sperm cells markedly reduced
	spermatids degenerated
	atrophy of tubules

Animal 2413 (killed on schedule)
==========

Macroscopy:	
Kidney	pelvis slightly dilated - bilateral
Skin	black spots on skin round nose and on forelimbs
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration
Kidney	slight bilateral hydronephrosis
Lung	lung showed generalized congestion with fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules

Animal 2415 (killed on schedule)
==========

Macroscopy:	
Lung	a few red spots on right upper and right lower lobes of lung
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Kidney	areas of interstitial nephritis
Lung	lung shows generalized fibrosis with areas of congestion
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Animal 2517 (killed on schedule)
==========

Macroscopy:	
Kidney	pelvis slightly dilated - unilateral
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Kidney	slight unilateral hydronephrosis
Lung	localized area of fibrosis
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Animal 2519 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of moderate cellular alteration in the cortex
Testis	sperm cells not present
	spermatids degenerated
	atrophy of tubules
	giant cells in tubules

Female dose-group 0 ppm

Microscopic observed organs : Adrenal Kidney Liver Lung Trachea

Animal 1102 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal areas of marked cellular alteration in the cortex

Animal 1104 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal zona fasciculata, foci of slight cellular alteration

Animal 1206 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal areas of marked cellular alteration in the cortex

Animal 1208 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal zona fasciculata, foci of marked cellular alteration

Animal 1310 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal zona fasciculata, foci of marked cellular alteration
Lung localized area of fibrosis

Animal 1312 (killed on schedule)
==========

Macroscopy: No abnormalities detected for any organ

Microscopy:
Adrenal areas of marked cellular alteration in the cortex
Lung areas of congestion
localized area of fibrosis

Animal 1414 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration

Animal 1416 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	localized area of fibrosis

Animal 1518 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Liver	a few small foci of mononuclear cells

Animal 1520 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration
Lung	alveolar wall sightly thickened

Female dose-group 10000 ppm

Microscopic observed organs : Adrenal Kidney Liver Lung Trachea

Animal 2102 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration

Animal 2104 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	localized area of fibrosis

Animal 2206 (found dead)
==========

Macroscopy:	
Animal	Found dead after 2 days
Genital area	fur stained
Liver	rough surface with white areas, pronounced lobular pattern
Skin	red crust on skin round left eye, nose and forelimbs
Thoracic cavity	contains clear fluid
Trachea	contents foamy
Thymus	single red spot
Microscopy:	
Adrenal	zona fasciculata, foci of marked cellular alteration
Kidney	convoluted tubules slightly dilated
Liver	areas of congestion - the rough surface is caused by alternating areas of congestion and necrosis being intersected by the periphery of the liver
	areas of necrosis
	centrilobular parenchyma cells swollen
	centrilobular parenchyma cells contain small vacuoles
Lung	marked oedema round vessels
	localized area of fibrosis
Trachea	no abnormalities detected - thus the foam seen at the post mortem examination does not appear to have originated from the tracheal mucosa

Animal 2208 (killed on schedule)
==========

Macroscopy:	
Lung	a few red spots on upper and middle lobes of right lung
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	alveolar wall moderately thickened
Trachea	slight inflammation in submucosa

Animal 2310 (found dead)
==========

Macroscopy:	
Animal	Found dead after six days Animal in rigor mortis. Neck hyperextended.
Abdominal cavity	contains clear red fluid
Genital area	fur stained
Heart	atrium dilated with blood
Liver	rough surface
Lung	diffuse red
Nose	clear red fluid round nose
Thoracic cavity	contains clear red fluid
Trachea	contains clear red fluid
Thymus	maculate
Microscopy:	
Liver	necrosis of centrilobular parenchyma cells - roughened surface noted at post mortem due to areas of necrotic tissue intersecting the surface
Lung	areas of congestion severe oedema round vessels
Trachea	no abnormalities detected - thus the fluid seen at post mortem has not originated from the trachea

Animal 2312 (killed on schedule)
==========

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex
Lung	marginal oedema round vessels

Animal 2414 (found dead)

Macroscopy:	
Animal	Found dead after 3 days
Eye	Left eye appears diffusely white
Liver	white spot on left lateral lobe
Lung	diffuse red
Skin	red crust on skin round both eyes, nose and both forelimbs
Thoracic cavity	contains clear fluid
Trachea	contents foamy
Thymus	red spots
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Liver	the left lateral lobe shows massive necrosis
	centrilobular parenchyma cells contain small vacuoles
Lung	areas of congestion
	alveolar wall moderately thickened
	moderate oedema round vessels
	localized area of fibrosis

Animal 2416 (killed on schedule)

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration

Animal 2518 (killed on schedule)

Macroscopy:	No abnormalities detected for any organ
Microscopy:	
Adrenal	zona fasciculata, foci of moderate cellular alteration
Lung	areas of congestion

Animal 2520 (killed on schedule)

Macroscopy:	
Thymus	maculate
Microscopy:	
Adrenal	areas of marked cellular alteration in the cortex

Table 1 **Haematology: Methods, references and units.**

White blood cell count (WBC) — 10^9/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; electric resistance detection.

Red blood cell count (RBC) — 10^{12}/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; electric resistance detection.

Hemoglobin (Hgb) — mmol/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; sodium lauryl sulfate-Hgb conjugate (SLS-Hb) at 540 nm.

Hematocrit (Hct) — l/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; Cumulative Pulse Height detection.

Mean corpuscular volume (MCV) — fl

Sysmex K1000 with PDA upgrade, automated hematology Analyser; computed from RBC and Hct.

Mean corpuscular haemoglobin (MCH) — fmol

Sysmex K1000 with PDA upgrade, automated hematology Analyser; Computed from RBC and Hgb.

Mean corpuscular haemoglobin concentration (MCHC) — mmol/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; Computed from Hct and Hgb.

Platelet (Plt) — 10^9/l

Sysmex K1000 with PDA upgrade, automated hematology Analyser; electric resistance detection.

Differential white blood cell count — 10^9/l and/or % of WBC

SOP TDS 048, TDS 051 and TDS 052. Staining with Maygrünwald-Giemsa

Table 2 **Clinical biochemistry: Methods, references and units**

Aspartate amino transferase (AST) U/l 30°C

Cobas Bio; SOP TDS 160; Roche set 7056. Kinetic UV test according to the recommendations of the IFCC.

1. International Federation of Clinical Chemistry (IFCC).
J.Clin. Chem. Clin. Biochem. 1986;24 497-510.
Approved Recommendations (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes.
2. Schlebusch H, Rick W, Lang H, Knedel M.
Normbereiche der Aktivitäten klinisch wichter enzym.
Dtsch. Med. Wochenschr. 1974;99 765-766.

Alanine amino transferase (ALT) U/l 30°C

Cobas Bio; SOP TDS 160; Roche set 7055. Kinetic UV test according to the recommendations of the IFCC.

1. International Federation of Clinical Chemistry (IFCC).
J.Clin. Chem. Clin. Biochem. 1986;24 481-495.
Approved Recommendations (1985) on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes.
2. Schlebusch H, Rick W, Lang H, Knedel M.
Normbereiche der Aktivitäten klinisch wichter enzym.
Dtsch. Med. Wochenschr. 1974;99 765-766.

Alkaline Phosphatase (ALP) U/l 30°C

Cobas Bio; SOP TDS 160; Boehringer set 158135. ; "Optimized standard method" conforming to the recommendations of the Deutsche Gesellschaft für Klinische Chemie.

1. Recommendations of the German Society for Clinical Chemistry.
Standardisation of methods for the estimation of enzyme activities in biological fluids.
Z. Klin. Chem. u. Klin. Biochem. 8, 658 (1970), 10, 281 (1972).

Glucose (Gluc) mmol/l

Cobas Bio;SOP TDS 160; Roche set 1097;Enzymatic method with hexokinase.

1. Bonder RJL, Mead DC.
Evaluation of glucose-6-phosphate dehydrogenase from leuconostoc mesenteroides in the hexokinase method for determining glucose in serum.
Clin. Chem. 1974; 20: 586-590

Albumine (Album) g/l

Cobas Bio; SOP TDS 160; Sigma set 631 bromcresolgreen method.

1. Corcoran R, Duran S.
Albumin determination by a modified bromcresolgreen method.
Clin. Chem. 1977; 23 :765-766
2. Savory J, Heintges M.G., Sonowane M. et all.
Measurement of total protein and albumin in serum with a centrifugal analyzer.
Clin. Chem. 1976; 22 :1102-1104

Urea-N (Urea) mmol/l

Cobas Bio; SOP TDS 160; Roche set 1537;Enzymatic UV test with urease and glutamate dehydrogenase.

1. Eisenwiener HG.
Fixed time determination of urea with the centrifichem system using a single reagent.
Arztl. Lab. 1976; 22: 53-59.
2. Eisenwiener HG.
Kinetic determination of urea with the LKB system.
Z. Klin. Chem. Klin. Biochem. 1976;14: 261-264.

Creatinine (Creat) µmol/l

Cobas Bio; SOP TDS 160; Roche set 1539 Method: buffered kinetic Jaffé reaction without deproteinization

1. Fabiny DL, Ertingshausen G.
Automated reaction-rate method for determination of serum creatinine with the centrifichem.
Clin. Chem. 1971; 17: 696-700.
2. Bartels H, Boehmer M.
Micro determination of creatinine.
Clin. Chim. Acta 1971; 32: 81-85.

Cholesterol (Chol) mmol/l

Cobas Bio; SOP TDS 160; Boehringer set 290319; CHOD-PAP method.

1. Siedel J., Hägele E. et al.
Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency.
Clin. Chem. 1983; 29: 1075.
2. Kattermann R., Jaworek D. et al.
Multicentre study of a new enzymatic method of cholesterol determination.
J. Clin. Chem. Biochem. 1984; 22: 245-251

Triglycerides (Trig) mmol/l

Cobas Bio; SOP TDS 160; Roche set Unimate 5 no.2216 method: Enzymatic colorimetric test with glycerol phosphate oxidase and 4-aminophenazone.

1. Fossati P., Prencipe L.
Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide.
Clin. Chem. 1982; 28:2077-2080
2. McGowan M.W., Artiss J.D. et all
A peroxidase-coupled method for the colorimetric determination of serum triglycerides.
Clin. Chem. 1983; 29:538-542

Calcium (Ca^{2+}) mmol/l

Cobas Bio; SOP TDS 160; Beckman set 683486; Reaction with methyl thymol blue.

1. Gindler EM, King JD.
Rapid colorimetric determination of calcium in biological fluids with methyl thymol blue.
Am. J. Clin. Path. 1972; 58: 376-382.

Inorganic phosphate (In.phos) mmol/l

Cobas Bio; SOP TDS 160; Roche set 1408; Direct phosphomolybdate reaction (kinetic) without deproteinization.

1. Daly J.A.,Ertingshausen G.
Direct method for determing inorganic phosphate in serum with the "Centrifichem".
Clin. Chem. 1972;18: 263-265.
2. Weissmann N.,Pileggi V.J.
in Henry R.J. et al.
Clinical Chemistry, Principles and Techniques, 2nd Edition, pp. 723-754, Harper&Row, Hagerstown/Md. 1974

Sodium (Na^{+}) mmol/l

Beckman Electrolyte-2, SOP TDS 191, Ion selective electrode, Indirect potentiometry.

1. North J.
New electrolyte assays.
A Technologist Primer 1970;7: 52-61.
2. Flores O, Chittenden C.
Ion selective electrode technology for sodium and potassium testing in the clinical laboratory.
Clinical Lab. Products 1981, 10/3:20-25.

Potassium (K^+) mmol/l

Beckman Electrolyte-2, SOP TDS 191, Ion selective electrode, Indirect potentiometry.

1. North J.
New electrolyte assays.
A Technologist Primer 1970;7: 52-61.

2. Flores O, Chittenden C.
Ion selective electrode technology for sodium and potassium testing in the clinical laboratory.
Clinical Lab. Products 1981; 10/3:20-25.

Oestradiol pg/ml

Analysis performed at the Clinical Biochemical Laboratory Leeuwarden, Leeuwarden, The Netherlands.
Sorin Biomedica S.p.A, 13040 Sallugia (VC) Italy P2210.
Method: RIA.

1. Mertens R, et al.
Evaluation of a radio-immuno-assay for estradiol in unextracted serum.
Clin. Chem. 29 (11); 1961; 1983.

Testosterone pg/ml

Analysis performed at the Clinical Biochemical Laboratory Leeuwarden, Leeuwarden, The Netherlands.
Byk-Sangtec Diagnostica GMbH & C0.KG, Dietzenbach, Germany.
Method: RIA.

Table 3 **Urine analysis: Methods, references and units**

Volume (Vol) ml

Volumetrically, SOP TDS 062.

Specific gravity (S.G.)

Refractive index, SOP TDS 060. Atago urine specific gravity refractometer

Sodium (Na^+) mmol or mmol/l

Beckman Electrolyte-2, SOP TDS 191, Ion selective electrode, Indirect potentiometry.

1. North J.
New electrolyte assays.
A Technologist Primer 1970;7: 52-61.
2. Flores O, Chittenden C.
Ion selective electrode technology for sodium and potassium testing in the clinical laboratory.
Clinical Lab. Products 1981; 10/3:20-25.

Potassium (K^+) mmol or mmol/l

Beckman Electrolyte-2, SOP TDS 191, Ion selective electrode, Indirect potentiometry.

1. North J.
New electrolyte assays.
A Technologist Primer 1970;7: 52-61.
2. Flores O, Chittenden C.
Ion selective electrode technology for sodium and potassium testing in the clinical laboratory.
Clinical Lab. Products 1981; 10/3:20-25.

Protein (Prot) mg and/or mg/l

Cobas Bio; SOP TDS 160; Pierce set 23200 Spectrophotometrical Coomassie Brilliant Blue Method.

1. Jongeneel J,
Eiwitbepaling in eiwitarme vloeistoffen, een evaluatie van de Coomassie Brilliant Blue Methode
Mededeling Ned. Ver. Klin Chem 1982; 4: 170-172.
2. Johnson JA, Lott JA.
Standardization of the Coomassie Blue Method for Cerebrospinalfluid proteins.
Clin. Chem. 1978; 24 1931 1933

Creatinine (Creat) µmol or µmol/l

Cobas Bio; SOP TDS 160; Roche set 1539,Method: buffered kinetic Jaffé reaction without deproteinization

1. Fabiny DL, Ertingshausen G.
Automated reaction-rate method for determination of serum creatinine with the centrefichem.
Clin. Chem. 1971; 17: 696-700.
2. Bartels H, Boehmer M.
Micro determination of creatinine.
Clin. Chim. Acta 1971; 32: 81-85.

γ-Glutamyl transferase (γ-GT) U/l 30°C or U/mmol Creat

Cobas Bio; SOP TDS 160; Boehringer set 158208; Kinetic method according to Szasz.

1. Szasz G., Persyn JP et al.
Effect of temperatures on enzyme activity and on the affinity of enzymes to their substrates.
Z. Klin. Chem. Klin. Biochem. 1974;12: 228.
2. Persyn JP, vd Slik W.
A new method for the determination of Y-glutamyl transferase in serum.
J. Clin. Chem. Clin. Biochem. 1976;14; 421.

N-Acetyl-ß-D-glucosaminidase (5-NAG) U/l 30°C or U/mmol Creat

Cobas Bio: SOP TDS 160; 4-methylumbelliferone as substrate.

1. Powell S.C., Scaro J. et all.
Assay of Urinary N-acetyl ß-glucosaminidase in a Centriugal Analyzer.
Clin. Chem. 1983; 29/10: 1717-1719.

Alkaline phosphatase (ALP) U/l 30°C or U/mmol Creat

Cobas Bio; SOP TDS 160; Boehringer set 158135. ; "Optimized standard method" conforming to the recommendations of the Deutsche Gesellschaft für Klinische Chemie.

1. Recommendations of the German Society for Clinical Chemistry.
Standardisation of methods for the estimation of enzyme activities in biological fluids.
Z. Klin. Chem. u. Klin. Biochem. 8, 658 (1970), 10, 281 (1972).

Osmolality (Osmo.) mosmol/kg

Gonotec Osmomat 030 cryoscopic osmometer. Method: Measurement of freezing point depression.

1. Henry J.B.
Clinical Diagnosis and Mangement by Laboratory methods.
W.B. Saunders company. Philadelphia, London and Toronto; 16 edition, 1979.

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Fluoride (F) mg/l

Analysis performed at the Institute of Biotechnology and Chemistry TNO, Zeist, The Netherlands.
Method: Gas Chromatography

1. Fresen J.A., Cox F.H. and Witter M.J.
The determination of fluoride in biological materials by means of gas chromatography.

Pharm.Weekblad 1968; 103: 909-914.

Urinary teststrip

Urotron RL9 Reflectance photometer with selective Light Emitting Diodes (LED's), SOP TDS 058

pH	5 - 9
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Leukocytes (Leu)	- till ++ [2]
Urotron^{R} RL9 (Combur^{9}-test, Boehringer set 759732)	
Protein (Pro)	- till +++ [2]
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Ketones (Ket)	- till ++ [2]
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Glucose (Glu)	- till +++ [2]
Urotron^{R} RL9 (Combur^{9}-test ,Boehringer set 759732)	
Bilirubin (Bil)	- till +++ [2]
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Blood (Ery)	- till +++ [2]
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Urobilinogen (Ubg)	- till +++ [2]
Urotron^{R} RL9 (Combur^{9}-test , Boehringer set 759732)	
Nitrite (Nit)	- till (+) [2]
Urotron^{R} RL 9 (Combur^{9}-test , Boehringer set 759732)	

---

2) - : negative
(+) : trace
\+ : small amount
++ : moderate amount
+++ : large amount

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SDS
SPECIAL DIETS SERVICES

EXACT COPY OF RAW DATA
DD: 6 Oct '93 Paraaf:

SPECIAL QUALITY CONTROL OF SMALL ANIMAL DIETS

CERTIFICATE OF ANALYSIS

PRODUCT: LAD 2 SQC
BATCH NO: 8612 PREMIX BATCH NO: 125/132
DATE OF MANUFACTURE: 25-NOV-92

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	9.4	%	Fluoride	23	mg/kg	1.0 mg/k
Crude Fat	3.5	%	Nitrate as NaNO3	26	mg/kg	1.0 mg/k
Crude Protein	22.6	%	Nitrite as NaNO2	2.4	mg/kg	1.0 mg/k
Crude Fibre	2.5	%	Lead	0.96	mg/kg	0.25 mg/k
Ash	5.2	%	Arsenic	0.47	mg/kg	0.2 mg/k
Calcium	0.91	%	Cadmium	0.13	mg/kg	0.05 mg/k
Phosphorus	0.69	%	Mercury	0.01	mg/kg	0.01 mg/k
Sodium	0.33	%	Selenium	0.24	mg/kg	0.05 mg/k
Chloride	0.43	%				
Potassium	0.69	%				
Magnesium	0.13	%	Total Aflatoxins	Non Detected	mcg/kg	1 mcg/kg each of B1,B2,G1
Iron	170	mg/kg				
Copper	10	mg/kg	Total P.C.B	Non Detected	mcg/kg	10.0 mcg/
Manganese	64	mg/kg	Total D.D.T	Non Detected	mcg/kg	1.0 mcg/
Zinc	60	mg/kg	Dieldrin	Non Detected	mcg/kg	1.0 mcg/
			Lindane	3	mcg/kg	1.0 mcg/
			Heptachlor	Non Detected	mcg/kg	1.0 mcg/
			Malathion	Non Detected	mcg/kg	20.0 mcg/
Vitamin A	15.9	iu/g	Total Viable Organisms x 1000	Non Detected	per grm	1000/g
Vitamin E	65	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores x 100	Non Detected	per grm	100/g
			Salmonellae Species	Non Detected	per grm	Absent in 20 grm
			Presumptive E coli	Non Detected	per grm	Absent in 20 grm
			E coli Type 1	Non Detected	per grm	Absent in 20 grm
			Fungal Units	100	per grm	Absent in 20 grm
			Antibiotic Activity	Non Detected		

Signed R S Fyvie
Dated 22/12/92

SOLVAY

hoofdafdeling kwaliteitsbeheer
j.w. lucasweg 2, 2031 be haarlem
postbus 5, 2060 ba bloemendaal
telefoon (023) 22 30 00
telefax (023) 22 32 12 *

analyserapport

nv pwn waterleidingbedrijf noord holland

Solvay Duphar B.V.
C.J. van Houtenlaan 36
1381 CP WEESP

rapportdatum	monsternummer	monsterdatum	monsterpunt	doorkiesnummer	bladnr
10-JUN-1993	102360	26-MAY-1993	WSPDUPLE	(023) 22 30 35	1

analyseprogram	monsterpuntomschrijving
DUPLEID	Duphar geb.WWO leiding

parameter nr	naam	eenheid	resultaat
1610	troebelingsgraad	FNE	<0.03
1680	elektrisch geleidend vermogen bij 20 °C	mS/m	50.7
1690	sommatie anionen	meq/l	5.43
1700	sommatie kationen	meq/l	5.39
1800	geur semikwantitatief door 1 persoon		0
1840	smaak kwalitatief bepaald door 1 persoon		afvezig
1910	temperatuur	°C	20.5
2010	opgelost zuurstof volgens Winkler	mg/l	7.4
2030	zuurstofverzadigingsperc vlg Winkler	%	83
2210	zuurgraad	pH	8.3
2410	vrij koolzuur	mg/l	0.0
2420	waterstofcarbonaat	mg/l	177
2440	carbonaat	mg/l	0.0
2470	chloride	mg/l	78
2500	sulfaat	mg/l	11
2610	natrium	mg/l	51
2640	kalium	mg/l	3.0
2670	calcium	mg/l	51
2700	magnesium	mg/l	6.6
2810	totale hardheid (berekend)	mmol/l	1.5
2840	tijdelijke hardheid	mmol/l	2.9
2850	evenwicht koolzuur	mg/l	-5.7
2860	agressief koolzuur	mg/l	-5.1
2870	overmaat waterstofcarbonaat	mg/l	0
2910	ammonium (als N)	mg/l	<0.02
2940	Kjeldahl stikstof	mg/l	0.8
2960	organisch gebonden stikstof	mg/l	0.8
2970	nitriet (als N)	mg/l	<0.010
2980	nitraat (als N)	mg/l	1.46
3000	ortho-fosfaat (als P)	mg/l	0.02
3010	totaal fosfaat (als P)	mg/l	0.17
3040	silicaat (als Si)	mg/l	4.3

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hoofdafdeling kwaliteitsbeheer
j.w. lucasweg 2, 2031 be haarlem
postbus 5, 2060 ba bloemendaal
telefoon (023) 22 30 00
telefax (023) 22 32 12

analyserapport *

nv pwn waterleidingbedrijf noord-holland

rapportdatum	monsternummer	monsterdatum	monsterpunt	doorkiesnummer	bladnr
10-JUN-1993	102360	26-MAY-1993	VSPDUPLE	(023) 22 30 35	2

parameter nr	naam	eenheid	resultaat
3110	ijzer	mg/l	<0.03
3210	mangaan	µg/l	<3
5010	totaal organisch koolstof	mg/l	2.5
5070	kaliumpermanganaat-index	mg/l	1.1
5100	UV-extinctie bij 254 nm	/m	4.5
5110	kleurgetal (Pt/Co-schaal)	mg/l Pt	<3
7010	kolonievormende eenheden 22 °C (mengpl.)	/ml	4
7020	kolonievormende eenheden 37 °C (mengpl.)	/ml	1
7120	bakteriën v/d coligroep voor bev (300ml)	/300ml	0
7140	thermotol bakt v/d coligr v bev (300ml)	/300ml	0

opmerkingen en conclusies

Chem. en Bact. in orde.

paraaf
hoofd KB

SOLVAY DUPHAR B.V.

Weesp, The Netherlands
Department of Toxicology
Int.Doc.No. 56345/43/93
Report No. S.9314
Issued April 1994
ADDENDUM

ADDENDUM
TO

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUORO-ETHANE (HFC 143) IN MALE AND FEMALE RATS

Authors: H.J.S. Koelman
P.J.M. Janssen
R.L.F. Dawes
M. de Haan

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Total number of pages is (iv) + 6

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Ref. 56345/43/93
ADDENDUM

STATEMENT OF GLP COMPLIANCE

With respect to the following study:

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS

I, the undersigned, hereby declare that this report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The study, performed in the Department of Toxicology of SOLVAY DUPHAR B.V., Weesp, The Netherlands, was conducted in accordance with:

- Good Laboratory Practice in the Testing of Chemicals, Good Laboratory Practice Principles, Organization for Economic Cooperation and Development (OECD), 1982, including all supplements published up to the starting date of this experiment.

With the exception of the following:
The fluoride measurements were performed at the Analytical Department of the Institute for Biotechnology at TNO, Zeist, The Netherlands; the oestradiol and testosterone analyses were performed at the Clinical Chemistry Laboratory Leeuwarden, Leeuwarden, The Netherlands. Both laboratories have never been inspected by the Section GLP of the Central Veterinary Public Health Inspectorate, nor have they been inspected by the Quality Assurance Unit of the sponsor.

Study director:

H.J.S. Koelman date: 27 April 1994

Head of the Department of Toxicology:

F.M.H. Debets date: 27 April 1994

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QA-STATEMENT

The following report has been audited by the Quality Assurance Unit of Solvay Duphar B.V.

Report No.	:	S.9314
Int.Doc.No.	:	56345/43/93
Title of the report	:	**ADDENDUM TO 14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS**
Authors	:	H.J.S. Koelman P.J.M. Janssen R.L.F. Dawes M. de Haan

The audit included the comparison of the individual data reported with the data recorded in notebooks, work sheets and other relevant papers.

This report has been accepted by the Quality Assurance Unit as being an accurate presentation of the individual findings of the study.

Date of inspection/audit

27 APR 94

Date of report to management

27 APR 94

C.J.M. van Gasteren
Head of the Quality Assurance Unit

Weesp, 27 APR 94

Weesp, The Netherlands
Department of Toxicology
Int.Doc.No. 56345/43/93
Report No. S.9314
Issued April 1994
ADDENDUM

ADDENDUM TO:

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUOROETHANE (HFC 143) IN MALE AND FEMALE RATS

1. SUMMARY

The basic report of this study was issued in February 1994. A study audit by the Section GLP of the Central Veterinary Public Health Inspectorate in the Netherlands, revealed some items that needed correction and/or clarification. The most important issues to be commented on were the interchange of two animals on one occasion during four hours of exposure (which was mentioned in the basic report, but the report did not explicitely indicate how this might have influenced the integrity and the conclusions of the study) and the fact that for three parameters the analyses were contracted out to laboratories that have not proven to be working in compliance with GLP.
The present addendum contains comments and clarification of the requested issues, as well as an adapted GLP Compliance Statement. In addition, some minor corrections are included that were noted during the additional data check required to prepare this addendum.

Weesp, April 1994

F.M.H. Debets
Head of Toxicology Department

H.J.S. Koelman
Author

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2. INTRODUCTION

The basic report of this study was issued in February 1994. A study audit by the Section GLP of the Central Veterinary Public Health Inspectorate in the Netherlands revealed some items that needed correction and/or clarification. The following issues were raised:

- Erroneous interchange of one control (no. 1520) and one test (no. 2518) animal: this error has been clearly stated in the report. However, no clear indication is given in the report as to how this error might have influenced the integrity and/or conclusions of the study.

- It was concluded that in the males fibrosis was a compound-related effect. However, in the females fibrosis was observed in three control and three test animals. Why has no statement been made on the females at all? And how can be proven that the fibrosis observed in control females was not caused by erroneous treatment due to additional interchanges?

- Analyses by non-GLP laboratories: The fluoride measurements were performed at the Analytical Department of the Institute for Biotechnology at TNO, Zeist, The Netherlands; the oestradiol and testosterone analyses were performed at the Clinical Chemistry Laboratory Leeuwarden, Leeuwarden, The Netherlands. Both laboratories have never been inspected by the Section GLP of the Central Veterinary Public Health Inspectorate, nor have they been inspected by the Quality Assurance Unit of the sponsor.

During the additional data check performed by the Study Director, some minor errors were noted in the report.

The present addendum contains comments on and clarification of the requested issues as well as corrections of the minor errors that were noted.

3. COMMENTS AND CLARIFICATION

As indicated in the basic report (page 11, fourth item), on 7 th June (day 5) female numbers 1520 (control) and 2518 (test) have erroneously been interchanged in the exposure chambers for about four hours. Immediately after discovery the two animals were replaced to their correct respective groups/exposure chambers.
In the report (page 11, last sentence) it was also stated that this error was not considered to have affected the integrity of the study. However, the report nor the raw data contained a proof of actions, checks or discussions on this issue to show how this could be concluded.

Indeed, no written information is available. Therefore a thorough review of the data and the procedures was performed by the Study Director.
The following items were checked and/or discussed:

- For all parameters the actual values and findings of the two interchanged animals were checked with the special attention to parameters that were concluded to be compound-related in one or both sexes. The following points are considered noteworthy:
  - During days 5-8, control animal no. 1520 lost 12 grams of weight, whereas the weight-gain for the other control animals varied from -5 to +1 gram; this weight loss might very well be an effect of the erroneous exposure to HFC 143. However, exclusion of this animal from the data on body weight and weight gain, would not have any influence on the conclusion of the study.
  - In the blood smear of animal no. 1520 one normoblast was noted. Not a single normoblast was recorded in any of the other males or females in the control group, and for 6 out of 10 males and 6 out of 7 females in the test group. This effect was considered to be compound-related. Normoblasts are occasionally seen in control animals. However, as in none of the other control animals normoblasts were found, this might well be a result of the erroneous treatment with HFC 143. The fact that normoblasts were not found in any other control animal, does not prove definitely that no other interchanges took place, but does reduce the chance that this has happened considerably.
  - No macroscopic findings were recorded at autopsy of animal no. 1520; at microscopy slight thickening of the alveolar wall was observed in the lungs and foci of marked cellular alteration in the zona fasciculata of the adrenal. The observation in the lung is not observed in any other control animal; it is, however, a common finding, and not related to any of the other lung findings (e.g., fibrosis). The observation in the adrenal was observed in several male and female control and test animals, and is therefore not considered to be compound-related.
  - For none of the other parameters the findings of animal nos. 1520 or 2518 were considered noteworthy.

- In the basic report it was concluded that in the males there was a treatment-related pulmonary alveolar fibrosis, as for 8 out of 10 HFC 143-treated animals this was recorded versus 0 out of 10 control males. In the females pulmonary alveolar fibrosis was observed in 3 out of 10 control and 3 out of 10 HFC 143-treated animals, including 2 decedents. The one control animal that was known to be erroneously exposed to HFC 143 for four hours did not show fibrosis, nor any other finding related to this (as indicated above). In order to estimate the probability of the observed fibrosis to be caused by possible unknown interchange of animals, all parameters of the three control females showing fibrosis (nos. 1310, 1312 and 1416) were carefully checked and compared with the other control and test animals. Special attention was given to those parameters where compound-related effects were observed in any of the sexes and the direction (increase or decrease) of the effect. With the exception of urinary ALP/creatinin, where the above three female animals had the three lowest values, and a compound-related decrease occurred, in no other parameter there was anything noteworthy. However, for this parameter animal no.1520, the animal which was known to have been exposed, did not show a lower ALP/creatinin value; in fact it had a relatively high value. Therefore, it is considered unlikely that this is an indication of possible interchange.

- The procedures that were followed and the checks that were built-in were re-discussed with the staff involved on the performance of the study. Although indeed no written detailed procedures other than the protocol were available and no records of checks performed were made, the Study Director and the performing staff are convinced that any interchange of animals would have been noted; if not, as was the case for animal no. 1520, during exposure, than at least after the 6-hour exposure period, when the animals were placed back into their cages.

- Based upon the above it was discussed whether or not any or all of the tables from the basic report should be adapted by excluding animal no. 1520 and possibly also no. 2518, and if so, whether statistical analysis should be repeated. From the above it was concluded that adaptation of the tables and additonal statistics would not influence the conclusions of the study. Therefore it was not considered worthwhile.

- As indicated in the adapted GLP Compliance Statement (see page ii of this addendum) the laboratories performing the fluoride and the hormone analysis have not been GLP-inspected as required for claiming GLP-compliance. However, for both laboratories the quality was checked and considered to be sufficient for the requested analysis. The actual raw data were sent to Solvay Duphar B.V. and checked and archived according to the internal GLP standards.

- Based upon the checks and discussions above it was concluded that the deviations in the study are not considered to have affected the integrity of the study.

- During the re-check of the report the following additional errors were observed:

- Page 14: The first paragraph of section 4.8 suggests that MCHC in the female was significantly increased; this is not the case; it was decreased. This paragraph should be adapted; but it has no effect on any other part of the report.
- Page 70: Typing error in relative lung weight values; all values should be "0.xxx"; for ten animals "1.xxx" is printed. As the report writing and the statistics have been performed based upon the correct data, this change has no effect on any other place in the report.

4. CONCLUSIONS

The following adaptations should be made to the basic report:

Page vi, STATEMENT OF GLP COMPLIANCE:

To be replaced by an adapted statement; see page ii of this addendum.

Page 11, last sentence, to be replaced by:

"After careful check of the data and the procedures followed it was concluded that none of the above mentioned deviations are considered to have affected the integrity of the study".

Page 14, section 4.8, first paragraph to be replaced by:

"Haematological examinations showed slight differences in the red cell indices of the treatment groups of both males and females when compared to the control group. Statistically significant differences were: increased MCV in both sexes, increased MCH in the males and decreased MCHC in the females".

Page 16, second paragraph to be replaced by:

"In the males there was a compound-related fibrosis of the alveolar walls, mainly localized, but in two animals (nos. 2413 and 2415) this was generalized. In the females this finding was observed in three control and three test animals, including two decedents. Even after careful check of the data and procedures, including check on known and possible unknown interchange of animals, it was concluded that there was no compound-related effects on the lungs in the females".

Page 70, last column (lung values):

For animal nos. 1208, 1310, 1312, 1414, 1416, 1518, 2208, 2312, 2415 and 2518 "1.xxx" should be replaced by "0.xxx".

As exclusion of the interchanged animals from the tables and statistics would not influence the outcome of the study, no further adaptations are considered necessary.

Despite the fact that two animals are known to have been interchanged once for four hours during exposure, after careful check of the data and the procedures it was concluded that the integrity of the study was not considered to have been affected.

SOLVAY DUPHAR B.V.

Weesp, The Netherlands
Department of Toxicology
Int.Doc.No. 56345/43/93
Report No. S.9314
Issued April 1994
ADDENDUM

ADDENDUM
TO

14-DAY INHALATION STUDY OF 1,1,2-TRIFLUORO-ETHANE (HFC 143) IN MALE AND FEMALE RATS

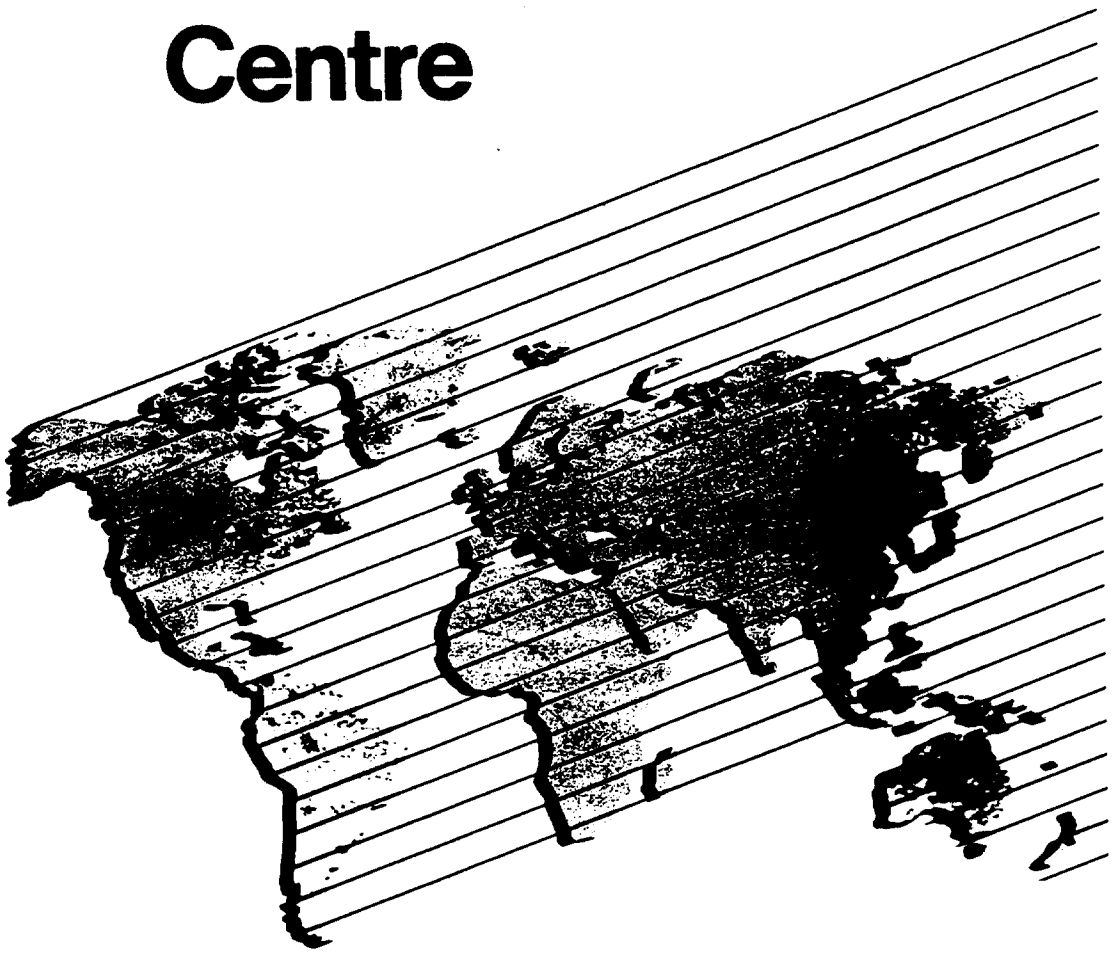
Authors: H.J.S. Koelman
P.J.M. Janssen
R.L.F. Dawes
M. de Haan

HRC Report

HFC 143
(TRIFLUOROETHANE)

28-DAY INHALATION TOXICITY STUDY IN RATS

Huntingdon Research Centre



Int.Doc.No. 56345/82/94
Report No. S.9410

HFC 143
(TRIFLUOROETHANE)

28-DAY INHALATION TOXICITY STUDY IN RATS

Sponsor

Solvay Duphar BV,
P.O. Box 900,
1380 DA Weesp
THE NETHERLANDS.

Testing facility

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued 21 December 1994

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FIGURES

TABLES

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APPENDICES

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

Derek W. Coombs, B.Sc.,
Study Director,
Huntingdon Research Centre Ltd.

21 December 1994
Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Date of reporting audit findings to the Study Director and HRC Management — 16 June 94

G.R. Keeble,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

20 December 1994
Date

QUALITY ASSURANCE STATEMENT

Inspections were made by the Quality Assurance Department of various phases of the study as conducted at HRC and described in this report. The dates on which the inspections were made and the dates on which findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection	Date of Reporting
Protocol Review	-	7 December 93
Pre-experimental Period	14 December 93	16 December 93
Experimental Period	21 December 93	21 December 93
	13 - 14 January 94	14 January 94
	20 January 94	20 January 94
	28 January 94	28 January 94

G.R. Keeble,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

20 December 1994
Date

RESPONSIBLE PERSONNEL

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Pearse C. Kieran, B.Sc. (Hons.),
Study Supervisor,
Division of Toxicology.

Derek W. Coombs, B.Sc.,
Study Director,
Division of Toxicology.

David Crook, B.Sc. (Hons.), Ph.D.,
Head of Department of Clinical Pathology.

William A. Gibson, B.Sc., (Medical Sciences),
Pathologist - Post Mortem Room,
Department of Pathology.

John M. Offer, B.Sc., Ph.D., M.I.Biol.,
Consultant Pathologist,
Department of Pathology.

Treatment-related findings in the testes	Air control		2250 ppm		4500 ppm		9000 ppm	
	T	R	T	R	T	R	T	R
Atrophic tubules lined only by Sertoli cells	0	0	2	5	3	5	4	4
Degenerate germ cells mainly spermatocytes	0	0	5	4	5	3	4	3
Reduction/absence of tailed spermatids	0	0	5	4	5	5	4	3
Reduction/absence of round spermatids	0	0	5	2	5	5	4	3
Reduction/absence of spermatocytes	0	0	5	2	5	5	4	3
Reduction/absence of spermatogonia	0	0	0	1	1	2	4	0
Multinucleate round spermatids	0	0	2	0	3	1	4	1
Multinucleate spermatocytes	0	0	0	0	0	0	2	0
Vacuoles in seminiferous epithelium	0	0	5	5	5	5	4	3
Total number of rats examined	5	5	5	5	5	5	5	5

T Terminal
R Withdrawal

Treatment-related findings in the epididymides	Air control		2250 ppm		4500 ppm		9000 ppm	
	T	R	T	R	T	R	T	R
Spermatozoa absent from caput	0	0	5	5	5	5	4	4
Spermatozoa absent from cauda	0	0	1	2	3	1	3	4
Reduced numbers of spermatozoa in cauda	0	0	2	3	2	4	1	0
Degenerate round germ cells	0	0	5	5	5	5	4	4
Total number of rats examined	5	5	5	5	5	5	5	5

T Terminal
R Withdrawal

Kidneys - Minimal casts in tubules at the corticomedullary junction in one male rat and two female rats receiving 9000 ppm at termination.

Incidental findings

All other microscopic findings seen in male and female rats at termination and withdrawal were considered to be of no toxicological importance.

No microscopic changes were seen which were considered related to the changes in red cell parameters recorded for rats receiving 4500 and 9000 ppm and for the reduced pH recorded in urinalysis for all male exposed groups and females receiving 4500 ppm.

SUMMARY

HFC 143 (1,1,2-trifluoroethane) was administered to rats by inhalation six hours a day, 5 days a week for 28 days. Three levels of exposure were used, 2250, 4500 and 9000 ppm and in addition a control group was exposed to air only.

Each group contained 10 male and 10 female rats, 5 of each sex originally scheduled for sacrifice at termination of exposures and following a 2 week withdrawal period. However, due to unscheduled deaths in the High dose group this sacrifice schedule was modified as a result and all surviving Group 4 females were terminated in Week 3. Details are found in the body of the report.

Data relating to clinical signs, bodyweight, food and water consumption, haematology, blood biochemistry, urinalysis, urinary fluoride, bone myelograms, organ weight, macroscopic and microscopic pathology were collected during the study.

No treatment-related effects on blood biochemistry and bone myelograms were present.

The following comments in relation to principal findings during the study are made in summary:

Mortality

Confined to Group 4 (9000 ppm) rats. A total of 8 rats (2 male and 6 females) died as a result of exposure.

Clinical signs

During exposure - reactions considered related to exposure to HFC 143 included:

Group 4 (9000 ppm) - hyperactivity; vertical jumping (popping) and convulsion, indicative of CNS involvement.

Groups 4 (9000 ppm) and 3 (4500 ppm) - breathing pattern irregularities; adoption of a hunched posture; eyes shut/half shut; piloerection, considered indicative of reaction to the presence of an irritant substance.

A convulsion seen in a single rat in Group 2 (2250 ppm) during the first exposure, may represent increased individual susceptibility to the test substance.

Between exposures - increased incidence of brown staining of the head was recorded in groups exposed to HFC 143 vapour. This was most obvious in male rats at 4500 ppm and 9000 ppm.

Adverse signs related to exposure at 4500 ppm and 9000 ppm included: pale extremities, lethargy, ataxia, exaggerated breathing, cold to touch, piloerection, poor grooming, body tremors and convulsions.

Chirukandath Gopinath, B.V.Sc., M.V.Sc., Ph.D., F.R.C.Path.,
Director of Pathology.

Bodyweight

Reduced gain by rats in Groups 3 (4500 ppm) and 4 (9000 ppm) over the period of exposure to HFC 143. Gain over the 2-week withdrawal period was considered normal.

Food consumption

Reduced consumption by rats in Groups 3 (4500 ppm) and 4 (9000 ppm) over the period of exposure to HFC 143.

Consumption during the 2-week withdrawal period was considered normal.

Water consumption

Reduced for Group 4 (9000 ppm) during the first week of exposure.

A slight increase in consumption by surviving female rats in Groups 3 (4500 ppm) and 4 (9000 ppm) was recorded over the period of exposures and a minimal increase in consumption by male rats in Groups 3 (4500 ppm) and 4 (9000 ppm) was evident during the withdrawal period.

Haematology

No abnormal values in samples removed from female rats in Group 4 (9000 ppm) killed during the third week of exposure.

Reduced packed cell volume, haemoglobin, red blood cell count, with increased mean corpuscular volume and mean corpuscular haemoglobin were present in rats from Groups 3 (4500 ppm) and 4 (9000 ppm) at the end of the 4-week period of exposures. The differences were slight, within expected normal range for rats of this age and strain but may be related to treatment.

Similar, but less obvious differences from control values were present in blood samples analysed at the end of the 2-week withdrawal period.

Urinalysis

Reduced pH was recorded in the urine of female rats in Group 4 (9000 ppm) at Week 3 and in all male groups exposed to HFC 143 at the end of the 4-week period of exposures.

The pH of urine removed at the end of the 2-week withdrawal period was similar for all male groups A slight reduction in the pH of female rats in Group 3 (4500 ppm) compared with control data was evident, but was not statistically significant.

Urinary inorganic fluoride

Increased urinary inorganic fluoride was recorded at the end of exposures in all groups exposed to HFC 143. No dose-relationship was present and analysis at the end of the withdrawal period revealed that urinary fluoride levels were still elevated, though to a lesser extent, in rats exposed at 4500 ppm, or higher. This is consistent with the clearance of the metabolic breakdown products of HFC 143 from the body.

Macroscopic pathology

Lung congestion, haemorrhage and oedema in a proportion of decedent rats was considered related to treatment, probably a direct lung effect of exposure.

Irregular cortical scarring with adhesion in the kidneys of 1 female rat in Group 4 (9000 ppm), killed during Week 3 may be related to treatment.

A dose-related reduction in the size of the testes was seen at the necropsy of male rats at the end of the 4-week period of exposures, and the end of 2-week withdrawal period. This effect was considered related to exposure to the vapour of HFC 143.

Organ weight

Severe, dose-related reduction in testes and epididymides weight, compared with control values, were recorded for male rats killed at the end of the 4-week period of exposure, and at the end of the 2-week withdrawal period. The reduction was considered related to exposure to the vapour of HFC 143.

Other differences recorded between test and control rats were considered not treatment-related.

Microscopic pathology

Treatment-related findings

Testes and epididymides - Degenerative changes in the testicular seminiferous epithelium with associated reduction or absence of spermatozoa and the presence of degenerate round germ cells in the epididymides were seen in all male rats from all HRC 143 treatment groups both at termination and at the end of the withdrawal period.

The principal testicular lesions was degeneration of spermatocytes with subsequent reduction or absence of spermatocytes, round and tailed spermatids and the presence of multinucleate spermatocytes or multinucleate round spermatids. In addition reduction or absence of spermatogonia was observed in a proportion of male rats. Varying numbers of atrophic seminiferous tubules lined only by Sertoli cells were present in a proportion of male rats at termination and in all rats at the end of the 2 week withdrawal period.

Vacuoles in the seminiferous epithelium were observed in the majority of rats. This probably indicates an effect upon Sertoli cells.

The atrophic seminiferous tubules lined only by Sertoli cells still present in withdrawal males represents irreversible testicular change within the study time scale. However, the small numbers of degenerated spermatocytes and multinucleate round spermatids may indicate some cessation of the primary damage following withdrawal. The other microscopic seminiferous epithelial changes may be expected to still be present after 2 weeks withdrawal as the normal cycle of the seminiferous epithelium is approximately 14 days in the laboratory rat.

Unscheduled deaths

There were eight unscheduled deaths in the 9000 ppm group. Haemorrhage in the lungs of three rats and vascular congestion and oedema in the lungs of five rats were considered to be factors contributory to death. These findings were considered to be treatment-related.

Other treatment-related findings seen only in the unscheduled deaths among rats receiving 9000 ppm included:

Thymus - Moderate involution in one male and two female rats.

Liver - Centrilobular hepatocyte necrosis in three female rats.

Conclusion

Toxic changes induced by inhalation of the vapour of HFC 143 were typified by acute lung effects at the high dose level of 9000 ppm resulting in significant mortality, and systemic toxicity to the testes at all exposure concentrations. A no adverse effect level was not established in this study.

ANIMALS AND MANAGEMENT

ANIMALS

One hundred (50 male and 50 female) Sprague-Dawley CD rats aged approximately 6 weeks were ordered from Charles River Ltd, Manston Road, Margate, Kent, England to arrive at Huntingdon Research Centre on 8 December 1993. One hundred and three (51 male and 52 female) rats were received on the stated date.

On the day of arrival 5 male and 5 female rats were killed and subjected to macroscopic examination as health check. The lungs, liver, kidneys, spleen and heart were preserved in buffered 10% formalin. No adverse signs were seen at health check necropsy.

All rats were arbitrarily selected and identified on a cage basis by a temporary mark on the tail. Allocation to 4 groups took place on 9 December 1994.

The rats were weighed and the individual bodyweights processed using a computer program which selected 90 rats (45 male and 45 female) for allocation to 5 groups, such that group mean bodyweights were approximately equalised. The rats were uniquely identified by numbers tattooed into the ear pinna.

The identification of individual rats in each group together with the initial allocation group mean bodyweights were as follows:

Group	Designation		Rat nos.		Group mean bodyweights (g)	
			♂	♀	♂	♀
1	Air control	- Main	1 - 5	41 - 45	172.4	141.6
		- Withdrawal	6 - 10	46 - 50		
2	Low dose	- Main	11 - 15	51 - 55	172.6	137.7
		- Withdrawal	16 - 20	56 - 60		
3	Intermediate dose	- Main	21 - 25	61 - 65	171.2	142.1
		- Withdrawal	26 - 30	66 - 70		
4	High dose	- Main	31 - 35	71 - 75	173.2	136.8
		- Withdrawal	36 - 40	76 - 80		
	Reserve		A - E	F - J	171.2	140.0
	Health check		81 - 85	86 - 90		

Reserve group rats were retained as potential replacements during the acclimatisation period and were killed when exposures commenced on 16 December 1993. Reserve rat F female was used to replace 51 female on 16 December 1993 due to unsatisfactory weight gain pre-exposure.

INTRODUCTION

This study undertaken at the Huntingdon Research Centre Ltd, Huntingdon, England, was designed to investigate the response of rats to repeated administration by inhalation of HFC 143 over a period of 4 weeks with a 2-week withdrawal phase.

Exposure levels were selected by the Sponsor.

On completion of the study, all data pertaining to the study, including preserved tissues and a copy of this report, were retained in the Archive Department of the Huntingdon Research Centre Ltd, Huntingdon, England.

Key dates in the study were:

Approval of protocol by:	
Study Director:	1 December 1993.
HRC Management:	1 December 1993.
Sponsor:	6 December 1993.
Arrival of rats:	8 December 1993.
Date of first exposure:	16 December 1993.
Dates of sacrifice:	
Surviving high dose females:	5 January 1994.
Main groups:	14 January 1994.
Withdrawal groups:	28 January 1994.

The study was designed to comply with the following guidelines:

EEC:	Guidelines 84/449/EEC/B8 and 92/69/EEC/B8.
OECD:	Guidelines for Testing of Chemicals No. 412.

TEST SUBSTANCE AND ADMINISTRATION

TEST SUBSTANCE

Code name:	HFC 143.
Chemical name:	1,1,2 trifluoroethane.
CAS no.:	430-66-0.
Presentation:	Liquefied compressed gas (BP 5°C).
Batch no.:	5128/271093.
Received from:	Solvay SA Usine de Tavaux BP1 F39500 Tavaux.
Receipt data:	29 November 1993.
Stability:	Stable for duration of study.
Expiry data:	October 1994.
Storage:	Ambient in the pressurised cylinders supplied.
Purity:	99.9%.
Vapour density:	3 (Relative to air at 25°C and 760 mmHg).

ADMINISTRATION

The rats were exposed, whole-body, to atmospheres containing HFC 143 for 6 hours a day, 5 days a week for 4 weeks.

Exposures commenced on a Thursday. Rats were exposed up to the day of the terminal kill. Due to technical reasons this involved an additional exposure on the first day of Week 5.

Control rats were similarly treated but no test gas was introduced into the exposure chamber.

ACCOMMODATION

The rats were housed singly in suspended cages with stainless steel sides and stainless steel mesh floors. Each cage measured 27 cm long, 27 cm wide and 20 cm high. Plastic trays, lined with absorbent paper were placed below each cage to collect animal excreta. The paper was changed daily and clean cages were introduced at intervals throughout the study.

Each group of rats was kept in a separate ventilated cabinet to prevent any possible cross-contamination between groups once exposures had commenced. The ventilated cabinets drew their air supply from the holding room. Exposure to the test material took place in a room adjacent to the room in which the animal holding cabinets were situated.

The temperature and relative humidity of the room were recorded using a Kent Clearspan TH 105 chart recorder (protocol limits were 21 ± 3°C and 55 ± 15%). The maximum and minimum values over the study period were as follows:

Holding room temperature: Minimum 19°C	Maximum 22°C
Holding room relative humidity: Minimum 42%	Maximum 62%

Lighting was controlled to give 12 hours light (0800 - 2000 hours) and 12 hours dark per 24 hours.

DIET

While in their cages all rats had free access to a weighed excess quantity of standard quality-controlled laboratory rat food (SDS Rat and Mouse No. 1 modified diet, Special Diets Services, Witham, Essex, England).

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet. Analyses were made on all batches of diet used to establish levels of basic nutrients and of specified substances and micro-organisms likely to have been present in the feed components and which, if in excess of specified amounts, might have had an undesirable effect on the test system. All batches of diet used conformed with the acceptable standards agreed by the Study Director and Head, Quality Assurance Department (see Appendix 1). The analytical data have been lodged in HRC Archives.

Tap water was available from moulded polypropylene water bottles at all times while the rats were in the cages. The water bottles were rinsed and refilled daily and thoroughly cleaned at intervals during the study.

There was no information available to indicate that any substance likely to influence the effect of the test substance could reasonably be expected to be present in the drinking water.

The results of the routine physical and chemical analyses of water at source (sampling point, Grafham Final Water) as conducted by the supplier, Anglian Water Services Ltd, have been made available to HRC. A list of the principal determinands is given in Appendix 1. The analytical data have been lodged in HRC Archives.

PROCEDURE

The rats were placed within the individual compartments of the exposure cages, the cages loaded into the exposure chambers and the chamber doors sealed.

Diluent airflows were switched on, chamber pressures were checked and adjusted, if necessary, to 10 mm of water below ambient.

Exposure commenced when the main test substance supply valve was opened. Following 6 hours of exposure the main test substance supply valve was closed. After approximately 25 - 48 minutes the rats were unloaded from the chambers and returned to their holding cages.

TARGET CONCENTRATIONS

The target concentrations of HFC 143 were as follows:

Group	Designation	Target concentration (ppm)
1	Air control	Air only
2	Low dose HFC 143	2250
3	Intermediate dose HFC 143	4500
4	High dose HFC 143	9000

A low dose of 2250 ppm was chosen, because this level is needed as a no (adverse) effect level for further development of the compound. A high dose of 9000 ppm was chosen to induce sufficient signs of toxicity without mortality. Data available at the Sponsor indicates that mortality occurs at 10000 ppm[5].

EXPOSURE SYSTEM AND PROCEDURE

TEST ATMOSPHERE GENERATION (Figure 1)

The test substance was metered under its own pressure from the cylinder into the inlet air of each chamber. The gas flow rates were controlled by in-line needle values and monitored by tapered tube flow meters (Meterate, Glass Precision Engineering Ltd, Hemel Hempstead, Hertfordshire, England). The flow rates required to achieve the target concentrations in a total gas/air flow of 100 litres per minute were as follows:

Group	HFC 143 flow rate (l/min)
2 (Low dose HFC 143)	0.225
3 (Int dose HFC 143)	0.450
4 (High dose HFC 143)	0.900

The control group received 100 l/min of air only.

The tapered tube flow meters used were recalibrated from air calibration by calculation knowing the density of HFC 143 vapour relative to air. Minor adjustments were made to gas flow rates during generation in order to maintain the desired concentrations.

EXPOSURE CHAMBERS (Figure 1)

The exposure chambers were constructed from stainless steel and glass and were of approximately 500 litres internal volume. The chambers were of square cross-section fitted with a pyramidal base and top. An extraction plenum was fitted in the base.

The chamber atmosphere was extracted by means of individual air handling units, each fitted with filters. A gate valve fitted in each extract line was used to adjust the pressure within each chamber to approximately 5 mm of water below that of the room.

Each chamber was fitted with ports for withdrawal of chamber air samples for analytical purposes. The atmosphere from the test group chambers was sampled from a middle port on the chamber side wall.

The rats were held within individual compartments of stainless steel wire mesh cages during exposure.

The test atmospheres were removed via a common exhaust system. The concentration of HFC 143 in this system was approximately 800 - 900 ppm. A second extract fan to provide additional diluent was mounted on the existing extract and the stack enlarged to eject the exhausted chamber air clear of the building.

CLINICAL OBSERVATIONS

CLINICAL SIGNS

During exposure

Clinical signs during exposure were recorded as a group response where all visible animals appeared to be responding similarly, or a proportion was affected. Group responses seen during exposure were not transferred to the individual clinical signs sheets, and are reported in the text of this report.

At other times

Animals were examined twice each day, usually prior to loading and immediately following unloading from the chamber on exposure days. An entry was made on the individual clinical signs sheets once each week even if abnormalities were not seen.

BODYWEIGHT

Each rat was weighed for allocation to groups, then twice weekly (Monday and Thursday), commencing 1 week (Thursday) before the start of dosing and continuing throughout the study.

In addition, the weight of each rat was recorded at necropsy.

FOOD CONSUMPTION

The quantity of food consumed by each rat was recorded twice weekly (Monday and Thursday) commencing one week (Thursday) prior to the start of exposures until the end of the study.

WATER CONSUMPTION

The quantity of water consumed by each rat was recorded daily throughout the study, commencing one week prior to the start of exposures.

EXPOSURE CHAMBER CONDITIONS

CHAMBER ANALYSED ATMOSPHERE CONCENTRATION OF HFC 143

The concentration of HFC 143 present in each chamber was monitored at least every 15 minutes throughout exposure by Miran 1A-CVF infra-red gas analysers. The concentrations obtained during each exposure were calculated from absorbance data at 30 - 40 minutes intervals.

The method of sampling and analysis is detailed in Appendix 2.

NOMINAL CONCENTRATION

Due to the large size and weight of the pressurised test substance cylinders, available equipment was not capable of measuring the amount of test substance delivered to the exposure chambers by weight loss from the cylinder. Such a measurement would not have provided information as to the amount of HFC 143 delivered to each individual chamber, therefore nominal concentrations were not determined during the course of the study.

During preliminary work the gas flow rate to each chamber was measured with an in-line wet type gas meter (Models DM3B and DM3D as appropriate. Alexander Wright and Co. (Westminster) Ltd,) and set to the flow rate as stated under TEST ATMOSPHERE GENERATION.

CHAMBER AIRFLOW

Diluent airflow was monitored continuously using tapered tube rotameters and recorded at 30-minute intervals throughout each exposure.

CHAMBER PRESSURE

The chamber internal pressure relative to ambient was monitored continuously by magnehelic pressure gauges and recorded at 30-minute intervals throughout exposure.

CHAMBER TEMPERATURE AND RELATIVE HUMIDITY

The wet and dry bulb temperatures of the thermohygrometer in each chamber were recorded at half-hourly intervals throughout each exposure. The chamber relative humidity was calculated from these data.

Units

Differential count (Diff) - standard microscopy of blood smear, stained with modified Wright's stain, counting 100 cells.

Neutrophils	(N)	$\times 10^3/mm^3$
Lymphocytes	(L)	
Eosinophils	(E)	
Basophils	(B)	
Monocytes	(M)	

Presence or absence of abnormal cells.

Thrombotest (TT) - Method of Owren, P.A. (Lancet 1959, ii, 754) — s

Reticulocyte count (Retic) - Method of Dacie, J.V., and Lewis, S.M. (Practical Haematology, 1966, 3rd edition p.28) — % (of red cells)

Microscopy of blood smear stained with modified Wright's stain to identify the following:

Polychromasia
Hypochromasia
Anisocytosis
Rouleaux
Separate film report

The results are reported according to the following convention:

NAD - No abnormality detected
1 - Slight
2 - Moderate
3 - Severe
4 - Gross

Abnormal cells recorded as a separate film report.

LABORATORY INVESTIGATIONS

Samples of blood and urine were collected from rats during Week 3 and at the end of Weeks 4 and 6 of the study.

Samples of blood and urine were collected from surviving female rats in Group 4 (High dose - 9000 ppm) immediately prior to sacrifice during Week 3.

Samples at the end of Week 4 were collected from surviving main group rats (urine from main and withdrawal rats) and at the end of Week 6 from surviving withdrawal group rats.

Urine samples were collected overnight from each individual animal. Each rat was housed in an individual metabolism cage without food for water for a period of approximately 16 hours. All rats were allowed access to water for a period of at least 1 hour prior to removal of blood samples.

HAEMATOLOGY

EDTA and sodium citrate (thrombotest) anticoagulants were used. The parameters used, together with the methods and units, were as follows:

	Units
Packed cell volume (PCV) Ortho ELT-1500	%
Haemoglobin (Hb) Ortho ELT-1500	g/dl
Red cell count (RBC) Ortho ELT-1500	$\times 10^6/mm^3$
Mean corpuscular haemoglobin concentration (MCHC) by calculation, Hb (g/dl) × 100 ÷ PCV (%)	%
Mean corpuscular volume (MCV) by calculation, PCV (%) × 10 ÷ RBC ($\times 10^6/mm^3$)	fl
Mean corpuscular haemoglobin concentration (MCH) by calculation, Hb (g/dl) × 10 ÷ RBC ($\times 10^6/mm^3$)	pg
Total white cell count (WBC Total)	$\times 10^3/mm^3$
Platelet count (Plts)	$\times 10^3/mm^3$

	Units
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' Reaction temperature 30°C	mU/ml
Gamma-Glutamyltransferase (γGT) Reaction temperature 30°C	mU/ml

URINALYSIS

The following estimations were performed using the appropriate methodology, as described below:

Volume	ml
Colour and appearance (visual assessment only)	
pH - by pH meter	
Specific Gravity (SG) - by Adago UR-1 refractometer	
Protein - Roche Cobas Centrifugal Analyser using modified method of Macart, M. and Gerbaut, L. *Clin. Chim. Acta.*, 1984, 141, 77).	mg/dl

Qualitative tests

Total reducing substances (TRS).....Clinitest
Glucose)
Ketones)
Bile pigments).....Multistix
Urobilinogen)
Haem pigments*)

Clinitest and Multistix are diagnostic reagents obtained from Ames Company, Stoke Poges, England and are used as qualitative indicators of analyte concentration. Results were reported according to the following convention:

0	=	negative
tr	=	'trace' of analyte
+	=	'small amount' of analyte
++	=	'moderate amount' of analyte
+++	=	'large amount' of analyte
++++	=	'very large amount' of analyte

BIOCHEMISTRY	**Units**
The blood was placed into proprietary blood collection vials containing lithium heparin anticoagulant. The blood was centrifuged for 3 minutes and the plasma analysed as below.	
The following parameter was analysed with a Roche Cobas centrifugal analyser, using the appropriate BCL test kit:	
Creatine phosphokinase (CPK), also known as 'creatine kinase' Reaction temperature 30°C	mU/ml
The following parameters will be analysed with an Hitachi 737 Clinical Chemistry analyser, using standard Hitachi 737 methodology:	
Total Protein	g/dl
Albumin (Alb)	g/dl
Globulin (Glob) - by subtraction, total protien (g/dl) - albumin (g/dl)	g/dl
Urea Nitrogen (Urea Nitr)	mg/dl
Alkaline phosphatase (AP) Reaction temperature 30°C	mU/ml
Total Bilirubin	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic Phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l
Cholesterol (Chol)	mg/dl
Glucose - hexokinase mediated	mg/dl
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' Reaction temperature 30°C	mU/ml

TERMINAL STUDIES

SACRIFICE

The original study protocol called for sacrifice of all Main group animals following 4 weeks of exposure. However, unscheduled deaths occurred in Group 4 (9000 ppm) during the course of the study. As a result of the number of deaths in female rats in this group, and in agreement with the Sponsor, it was decided to sacrifice the 4 female survivors during Week 3 of the study.

All remaining, surviving Main group rats were sacrificed following 4 weeks of exposure.

Withdrawal group rats were held unexposed for a further 2 weeks and then sacrificed.

The rats were killed by exsanguination from the brachial arteries following anaesthesia induced by intraperitoneal injection of pentobarbitone sodium.

BONE MARROW

When anaesthetised, samples of marrow were removed from the tibiae of all rats (except unscheduled deaths). Smears were made on clean glass slides, air dried and fixed in methanol prior to staining.

The total cell count, together with erythroid and myeloid cell counts were made and the myeloid/erythroid (M/E) ratios calculated.

MACROSCOPIC PATHOLOGY AND ORGAN WEIGHT ANALYSIS

The macroscopic appearance of all tissues was noted and the following organs dissected free from each animal and weighed:

adrenals	lungs	epididymides (left)
kidneys	testis (left)	epididymides (right)
liver	testis (right)	

For haem pigments this degree of differentiation is not possible and the results were reported as negative (0) or positive (+) only.

INORGANIC FLUORIDE ANALYSIS

Urine samples from all surviving rats at the end of Weeks 4 and 6 were deep-frozen and despatched for analysis of inorganic fluoride by ion specific electrode to:

Butterworth Laboratories,
54 - 56 Waldegrave Road,
Teddington,
Middlesex,
TW11 8LG.

The samples were received at Butterworth Laboratories on 2 February 1994.

Prior to the collection of overnight urine samples during Week 3 (surviving female rats in Group 4) and at the end of Weeks 4 (Main group rats) and 6 (withdrawal group rats) the individual metabolism cages were washed with a solution of nitric acid in deionised water (1 : 20 v/v) to remove any fluoride contamination that may have been present. This was followed by rinsing with deionised water followed by air drying.

The glass urine separators and scintillation vials used for collection and storage were also rinsed in nitric acid solution and rinsed with deionised water. The separators and vials were heat dried in an oven.

The ion-specific electrode methodology is presented in Appendix 24.

STATISTICAL ANALYSIS

All statistical analyses were carried out separately for males and females.

For all parameters the analyses were carried out using the individual animal as the basic experimental unit. Food and water consumption data were analysed using cumulative intake. Bodyweight data were analysed using weight gains. The following sequence of statistical tests was used for food and water consumption, bodyweight, organ weight and clinical pathology data:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%) the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:

Bartlett's test (1) was applied to test for heterogeneity of variance between treatments; where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.

Except for pre-exposure data, analyses of variance were followed by Student's 't' test and Williams' test (3) for a dose-related response, although only Williams' test was reported. The Kruskal-Wallis analyses were followed by Shirley's test (4), the non-parametric equivalent of the 't' and Williams' tests.

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence. For organ weight data, the final bodyweight was used as covariate in an attempt to allow for differences in bodyweight which might affect the organ weights.

FIXATION OF TISSUES

Samples, or the whole of the following organs/tissues, together with any macroscopically abnormal entities were preserved in neutral buffered 10% formalin. The eyes were preserved in Davidson's fixative. The lungs were infused and the nasal cavities flushed with fixative prior to immersion.

[a]adrenals
oesophagus
stomach (glandular and non-glandular)
duodenum
jejunum
ileum
caecum
colon
rectum
animal identification mark
aorta
brain
eyes
femur with joint (for bone and marrow *in situ*)
[a]gross abnormalities
[a]heart
[a]kidneys
[a]larynx
[a]liver
[b]lungs (all lobes and mainstem bronchi)
lymph nodes (cervical, mesenteric and tracheobronchial)
mammary gland
[a]nasal passages (head for rostral and caudal nasal cavities)
optic nerve
ovaries
pancreas
pharynx
pituitary
prostate
salivary gland
sciatic nerve
seminal vesicles
skeletal muscle (thigh)
skin
spinal column
spinal cord (cervical, thoracic and lumbar)
spleen
sternum
[c]testes* (with epididymides)*
thymus
thyroid (with parathyroids)
tongue
[a]trachea (including bifurcation)
ureter
urinary bladder
uterus (corpus and cervix)
vagina

* Right and left preserved separately in Bouin's fixative

MICROSCOPIC EXAMINATION

Light microscopic examination was performed on 4 μm thick sections, stained with haematoxylin and eosin, of those tissues in the table above marked as follows:

a Rats from Main groups 1 (Air control) and 4 (High dose HFC 143)
b All Main group rats
c All surviving main and withdrawal group rats. Examination was performed on longitudinal 4 μm thick sections of the epididymides, stained with haematoxylin and eosin, and transverse 4 μm thick sections of the testes, stained with Periodic Acid, Schiff's reagent (PAS) and haematoxylin

Light microscopic examination with reference to the stages of spermatogenesis was made.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Analysed concentration of HFC 143

The data are presented as follows:

Table 1 - exposure mean values
Appendix 3 - individual values

The data are summarised below:

Group	Study mean concentration of HFC 143 (ppm)	
	Target	Analysed
2 (Low dose)	2250	2226 (SD 27.0)
3 (Inter dose)	4500	4443 (SD 102.9)
4 (High dose)	9000	8920 (SD 83.8)

The analysed concentrations were in close agreement with target levels.

Chamber temperature and relative humidity

The data are presented as follows:

Table 2 - exposure mean values

The data are summarised below:

Group	Study mean	
	Temperature (°C)	Relative humidity (%)
1 (Air control)	23.4	31
2 (Low dose)	23.5	42
3 (Inter dose)	22.4	26
4 (High dose)	21.9	30

The differences between groups are considered not to have affected the results of the study.

Chamber distribution of HFC 143 vapour

The results of sampling at 3 points within each chamber on two occasions during the study are presented in Table 3.

The distribution within each chamber was considered acceptable.

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Between exposures - No treatment-related signs were seen for male rats in Groups 1 (Air control) and 2 (2250 ppm). Brown staining of the head was seen in a proportion of female rats in these groups, more obvious in Group 2 (2250 ppm).

Brown staining of the head was noted in a proportion of male and female rats in Groups 3 (4500 ppm) and 4 (9000 ppm) most obvious at 4500 ppm and in female rats.

Signs persisting between exposures, considered associated with the adverse effect of exposure were seen in a proportion of male rats in Group 4 (9000 ppm), in a proportion of female rats in Group 3 (4500 ppm) and in all female rats in Group 4 (9000 ppm). The signs included:

pale extremities, lethargy, ataxia, exaggerated breathing, cold to touch, piloerection, poor grooming, body tremors and convulsions.

Deaths due to exposure to HFC 143 were confined to 2 male and 6 female rats in Group 4 (9000 ppm).

Bodyweight

The data are presented as follows:

Figure 3 - group mean values
Table 5 - group mean values
Appendix 5 - individual values

Reduced bodyweight gain was recorded for male and female rats in Groups 3 (4500 ppm), not statistically significant and 4 (9000 ppm) during the period of exposures. The reduction in bodyweight gain by female rats in Group 3 (4500 ppm) was slight.

The differences achieved statistical significance when compared with control weight gain in Group 4 (9000 ppm) only, male rats, $P < 0.01$. (All Group 4 (9000 ppm) female rats were dead by Week 3 of the study so no comparison at this time point was possible.)

No treatment-related reduction in bodyweight gain was apparent during the 2-week withdrawal period.

Food consumption

The data are presented as follows:

Table 6 - group mean values
Appendix 6 - individual values

Reduced food consumption was recorded for male and female rats in Groups 3 (4500 ppm) and 4 (9000 ppm) during the period of exposures. The reduction in food consumption in Group 3 (4500 ppm) rats was confined to Week 1. When cumulative values at Week 4 were compared with control consumption the differences achieved statistical significance for male rats in Group 4 (9000 ppm), $P < 0.01$ and for female rats in Group 3 (4500 ppm); $P < 0.05$. (All Group 4 (9000 ppm) female rats were dead by Week 3 of the study so no comparison at this time point was possible.)

No treatment-related differences were seen over the 2-week withdrawal period.

CLINICAL OBSERVATIONS

Mortality

Eight rats (2 male and 6 female) in Group 4 (High dose), were found dead or were sacrificed due to moribund condition during the study. These deaths were considered directly related to exposure to HFC 143.

The pattern of unscheduled deaths in Group 4 (High dose) was as follows:

Rat no./sex	Date of death	Week	Comment
75♀	18 December 1993	1	Found dead in holding cage
37♂	19 December 1993	1	Found dead in holding cage
79♀	19 December 1993	1	Found dead in holding cage
76♀	20 December 1993	1	Found dead in holding cage
34♂	22 December 1993	1	Found dead in holding cage
78♀	22 December 1993	1	Moribund condition/sacrificed
80♀	22 December 1993	1	Found dead in holding cage
77♀	3 January 1994	3	Found dead in holding cage

As a result of the high proportion of deaths in female rats of this group, exposure was terminated on 4 January 1994 and the remaining 4 female rats (nos 71, 72, 73 and 74) subjected to terminal necropsy on 5 January 1994.

The remaining 8 male rats in Group 4 (High dose) continued exposure to the end of the study without further deaths occurring.

Clinical signs

The data are presented as follows:

Table 4 - during exposure
Appendix 4 - between exposures, individuals

During exposure - Signs considered consistent with CNS effects were seen in a proportion of rats in Group 4 (9000 ppm) including hyperactivity, vertical jumping (popping) and convulsions.

Other signs considered treatment-related, seen in a proportion of rats in Group 3 (4500 ppm) and 4 (9000 ppm) included exaggerated/slow breathing movements, eyes closed/half-closed, adoption of a prone/hunched posture and piloerection.

In general signs were most obvious in exposures carried out following a weekend period.

A single instance of convulsion in a rat in Group 2 (2250 ppm), followed by prone posture with slow breathing may represent an increased susceptibility to the test substance in this rat.

At the end of the withdrawal period blood samples removed for red blood cell parameter analysis only, showed differences achieving statistical significance when compared with control data as follows:

Reduced red blood cell count, $P<0.01$ in male rats in Group 4 (9000 ppm).

Increased mean cell volume, $P<0.01$ and mean corpuscular haemoglobin; $P<0.01$, for male rats in Groups 2 (2250 ppm) and 4 (9000 ppm) and in both sexes in Group 3 (4500 ppm).

Blood biochemistry

The data are presented as follows:

Table 10 - group mean values at the end of the period exposures
Appendix 11 - individual values; rats killed 5 January 1994 (Week 3)
Appendix 12 - individual values at the end of the period of exposures

Data from blood samples removed from Group 4 (9000 ppm) female rats during Week 3 did not indicate any treatment-related abnormality.

Differences achieving a degree of statistical significance compared with control data in samples removed at the end of exposures (Week 5) were as follows:

Decreased potassium values in female rats in Groups 2 (2250 ppm) and 3 (4500 ppm); $P<0.01$.

This difference was considered not treatment-related.

Urinalysis

The data are presented as follows:

Table 11 - group mean values at the end of the period of exposures
Table 12 - group mean values at the end of the withdrawal period
Appendix 13 - individual values; rats killed 5 January 1994 (Week 3)
Appendix 14 - individual values at the end of the period of exposures
Appendix 15 - individual values at the end of the withdrawal period

Increased acidity (reduced pH values) was recorded in the urine of Group 4 (9000 ppm) female rats during Week 3. This was considered a treatment-related effect.

At the end of exposure differences achieving a degree of statistical significance compared with control data were as follows:

Decreased pH values in the urine of male rats in Groups 2 (2250 ppm) and 4 (9000 ppm) and in male and female rats in Group 3 (4500 ppm). This effect was considered treatment-related

Water consumption

The data are presented as follows:

Table 7 - group mean values
Appendix 7 - individual values

Reduced water consumption was recorded during the first week of exposures (notably the first few days) for male and female rats in Group 4 (9000 ppm) that died or were sacrificed (2 male and 5 female rats in Week 1). Subsequently, in surviving male and female rats a slight increase in the cumulative consumption by surviving male rats in Group 4 (9000 ppm) and male and female rats in Group 3 (4500 ppm) was evident over the study period. Comparison with control consumption achieved statistical significance only for female rats in Group 3 (4500 ppm); $P < 0.05$.

A minimal increased consumption was present during the 2-week withdrawal phase for male rats in Groups 3 (4500 ppm) and 4 (9000 ppm).

LABORATORY INVESTIGATIONS

Haematology

The data are present as follows:

Table 8 - group mean values at the end of the period of exposures
Table 9 - group mean values at the end of the withdrawal period
Appendix 8 - individual values, rats killed 5 January 1994 (Week 3)
Appendix 9 - individual values, at the end of the period of exposures
Appendix 10 - individual values, at the end of the withdrawal period

Data from blood samples removed from Group 4 (9000 ppm) female rats during Week 3 are difficult to interpret in isolation but no abnormal values were observed.

At the end of exposures differences achieving a degree of statistical significance compared with control data were as follows:

Reduced packed cell volume ($P < 0.05$), haemoglobin ($P < 0.05$), red blood cell count ($P < 0.01$), with increased mean cell volume; $P < 0.01$ and mean corpuscular haemoglobin, ($P < 0.05$) for male rats in Group 3 (4500 ppm) and 4 (9000 ppm).

Reduced haemoglobin $P < 0.05$ was recorded for female rats in Group 3 (4500 ppm).

An increased incidence of anisocytosis was present in female rats in Group 2 (2250 ppm) and 3 (4500 ppm) compared with control data.

No other treatment-related findings were seen at necropsy at Week 5 or 7 that were considered treatment-related.

Bone myelograms

The data are presented as follows:

Table 19 - group mean values at the end of the period of exposures
Table 20 - group mean values at the end of the withdrawal period
Appendix 18 - individual values at the end of the period of exposures
Appendix 19 - individual values at the end of the withdrawal period

No treatment-related effects were seen in bone marrow removed from rats at the end of the 4-week period of exposure; or at the end of the 2-week withdrawal period.

All values obtained were considered to be within expected normal ranges for these data.

Organ weights

The data are presented as follows:

Table 21 - group mean values at the end of the period of exposures
Table 22 - group mean values at the end of the withdrawal period
Appendix 20 - individual values; rats killed 5 January 1994 (Week 3)
Appendix 21 - individual values at the end of the period of exposures
Appendix 22 - individual value at the end of the withdrawal period

Data obtained from female rats in Group 4 (9000 ppm) killed during Week 3 of the study revealed no treatment-related changes.

At the end of 4 weeks of exposure, Week 5, differences achieving a degree of statistical significance compared with control data were as follows:

Reduced liver weights for male rats in Groups 2 (2250 ppm), 3 (4500 ppm) and 4 (9000 ppm); $P<0.05$. Increased liver weight for female rats in Group 3 (4500 ppm); $P<0.05$.

Reduced testes weights for male rats in Groups 2 (2250 ppm), 3 (4500 ppm) and 4 (9000 ppm), $P<0.01$.

Reduced epididymides weights for male rats in Groups 2 (2250 ppm); $P<0.05$, 3 (4500 ppm), $P<0.05$ (left) - $P<0.01$ (right), and 4 (9000 ppm); $P<0.01$.

The reduction in testes and epididymides weight was dose-related and considered related to treatment with HFC 143.

Liver weight differences were inconsistent between the sexes and considered unlikely to be treatment related.

At the end of the withdrawal period, Week 7, differences achieving a degree of statistical significance compared with control data were as follows:

Increased liver weights for female rats in Groups 2 (2250 ppm) and 3 (4500 ppm); $P<0.05$.

Reduced testes and epididymides weights for male rats in Group 2 (2250 ppm), 3 (4500 ppm) and 4 (9000 ppm); $P<0.01$.

In the absence of a liver weight effect in male rats, the slight increase in liver weights of female rats exposed to HFC 143 is equivocal and is unlikely to be a treatment-related effect.

The dose-related reduction in testes and epididymides weights of male rats exposed to HFC 143 is considered a treatment-related effect.

Microscopic pathology

The data are presented as follows:

Table 23 - incidence summary; unscheduled deaths
Table 24 - incidence summary; termination of exposures
Table 25 - incidence summary; at the end of the withdrawal period
Appendix 23 - individual findings

Treatment-related findings

Testes and epididymides - Degenerative changes in the testicular seminiferous epithelium with associated reduction or absence of spermatozoa and the presence of degenerate round germ cells in the epididymides were seen in all male rats from all HRC 143 treatment groups both at termination and at the end of the withdrawal period.

The principal testicular lesions was degeneration of spermatocytes with subsequent reduction or absence of spermatocytes, round and tailed spermatids and the presence of multinucleate spermatocytes or multinucleate round spermatids. In addition reduction or absence of spermatogonia was observed in a proportion of male rats. Varying numbers of atrophic seminiferous tubules lined only by Sertoli cells were present in a proportion of male rats at termination and in all rats at the end of the 2 week withdrawal period.

Vacuoles in the seminiferous epithelium were observed in the majority of rats. This probably indicates an effect upon Sertoli cells.

The atrophic seminiferous tubules lined only by Sertoli cells still present in withdrawal males represents irreversible testicular change within the study time scale. However, the small numbers of degenerated spermatocytes and multinucleate round spermatids may indicate some cessation of the primary damage following withdrawal. The other microscopic seminiferous epithelial changes may be expected to still be present after 2 weeks withdrawal as the normal cycle of the seminiferous epithelium is approximately 14 days in the laboratory rat.

At the end of the withdrawal period apart from a slight reduction of pH in female Group 3 (4500 ppm) rats, which was not statistically significant compared with control data, there were no treatment-related differences.

Urinary inorganic fluoride

The data are presented as follows:

Table 13 - group mean values at the end of the period of exposures
Table 14 - group mean values at the end of withdrawal period
Appendix 16 - individual values at the end of the period of exposures
Appendix 17 - individual values at the end of the withdrawal period

Increased urinary fluoride concentration and total fluoride output were recorded for all groups exposed to HFC 143 at the end of the period of exposures; $P<0.01$. A dose-related effect was not present.

At the end of the withdrawal period increased total urinary fluoride was present for female rats in Groups 2 (2250 ppm) and 3 (4500 ppm) and for male rats in Groups 3 (4500 ppm) and 4 (9000 ppm). However, the relative increase compared with results obtained at the end of the series of exposures was lower and only for male rats in Group 4 (9000 ppm) did the difference achieve statistical significance compared with control data.

TERMINAL STUDIES

Macroscopic pathology

The data are presented as follows:

Table 15 - unscheduled deaths
Table 16 - rats killed 5 January 1994 (Week 3)
Table 17 - termination of exposures
Table 18 - at the end of the withdrawal period
Appendix 23 - individual findings

The presence of fluid in the thoracic cavity and congestion of the brain, inguinal lymph nodes, thymus and lungs were seen in a proportion of rats that died or were killed as a result of exposure. As the majority of rats were found dead in their holding cages, and the exact time of death not established, the changes observed may be agonal in origin.

The presence of kidney abnormalities; pale areas, irregular cortical scarring and adhesions in 1 of the 4 female rats in Group 4 (9000 ppm) killed during Week 3 of the study may be related to exposure.

A dose-related reduction in size of the testes and epididymides of a proportion of male rats in all groups exposed to HFC 143 was recorded at the end of the 4-week exposure period and at the end of the 2-week withdrawal period. This was considered directly related to exposure to HFC 143.

Treatment-related findings in the testes	Air control		2250 ppm		4500 ppm		9000 ppm	
	T	R	T	R	T	R	T	R
Atrophic tubules lined only by Sertoli cells	0	0	2	5	3	5	4	4
Degenerate germ cells mainly spermatocytes	0	0	5	4	5	3	4	3
Reduction/absence of tailed spermatids	0	0	5	4	5	5	4	3
Reduction/absence of round spermatids	0	0	5	2	5	5	4	3
Reduction/absence of spermatocytes	0	0	5	2	5	5	4	3
Reduction/absence of spermatogonia	0	0	0	1	1	2	4	0
Multinucleate round spermatids	0	0	2	0	3	1	4	1
Multinucleate spermatocytes	0	0	0	0	0	0	2	0
Vacuoles in seminiferous epithelium	0	0	5	5	5	5	4	3
Total number of rats examined	5	5	5	5	5	5	5	5

T Terminal
R Withdrawal

Treatment-related findings in the epididymides	Air control		2250 ppm		4500 ppm		9000 ppm	
	T	R	T	R	T	R	T	R
Spermatozoa absent from caput	0	0	5	5	5	5	4	4
Spermatozoa absent from cauda	0	0	1	2	3	1	3	4
Reduced numbers of spermatozoa in cauda	0	0	2	3	2	4	1	0
Degenerate round germ cells	0	0	5	5	5	5	4	4
Total number of rats examined	5	5	5	5	5	5	5	5

T Terminal
R Withdrawal

Kidneys - Minimal casts in tubules at the corticomedullary junction in one male rat and two female rats receiving 9000 ppm at termination.

Incidental findings

All other microscopic findings seen in male and female rats at termination and withdrawal were considered to be of no toxicological importance.

No microscopic changes were seen which were considered related to the changes in red cell parameters recorded for rats receiving 4500 and 9000 ppm for the reduced pH recorded in urinalysis for all male exposed groups and females receiving 4500 ppm.

Unscheduled deaths

There were eight unscheduled deaths in the 9000 ppm group. Haemorrhage in the lungs of three rats and vascular congestion and oedema in the lungs of five rats were considered to be factors contributory to death. These findings were considered to be treatment-related.

Other treatment-related findings seen only in the unscheduled deaths among rats receiving 9000 ppm included:

Thymus - Moderate involution in one male and two female rats.

Liver - Centrilobular hepatocyte necrosis in three female rats.

Conclusion

Testicular changes with associated epididymal changes in male rats treated with HFC 143 at all exposure levels both at termination and at the end of a 2 week withdrawal period. These changes were centred upon degeneration of spermatocytes and damage to Sertoli cells.

Mineral casts in renal tubules in a small number of male and female rats receiving 9000 ppm at termination.

Haemorrhage or vascular congestion and oedema in the lungs of unscheduled deaths from the 9000 ppm group.

Thymic involution in a small number of female unscheduled deaths in the 9000 ppm group.

Centrilobular hepatocyte necrosis in a small number of female rats which were unscheduled deaths in the 9000 ppm group.

DISCUSSION

Groups of rats were exposed by inhalation, 6 hours a day, 5 days a week for 4 weeks to chamber concentrations of 2250, 4500 and 9000 ppm HFC 143 vapour.

Treatment-related signs recorded during exposure included those considered associated with reaction to the presence of an irritant (eyes shut/half-shut; hunched posture; irregular breathing pattern), seen in Groups 3 (4500 ppm) and 4 (9000 ppm). Additionally for rats in Group 4 (9000 ppm) signs indicating involvement of the central nervous system were also seen (convulsion; hyperactivity; vertical jumping-popping). Treatment-related clinical signs recorded between exposures indicated systemic toxicity (lethargy; ataxia; pale extremities, tremors; cold to touch; convulsion). The signs were more obvious in female rats in Group 4 (9000 ppm). As a result of the toxicity of HFC 143, 8 rats (2 male and 6 female) died or were sacrificed, and exposure of the remaining female rats in Group 4 (9000 ppm) terminated during Week 3 of the study.

Microscopic examination of organs/tissues noted to be macroscopically abnormal at necropsy of decedent rats indicated that acute lung damage (haemorrhage and vascular congestion) was the major causative factor in the death of these rats.

In surviving Group 4 (9000 ppm) and 3 (4500 ppm) rats, reduced bodyweight gain and food consumption was evident over the study period. The effects at 4500 ppm were considered slight.

At 9000 ppm reduced water consumption was evident over the first 2 - 3 days of Week 1 of exposure and persisted in rats that died or were sacrificed. Surviving rats showed increased water consumption following this reduced intake phase. This is in agreement with the findings in a previous 14 day inhalation study, in which male and female rats were exposed to 10000 ppm HFC 143[5].

To a lesser extent increased water consumption was seen for male and female rats exposed to 4500 ppm over the study period. This slight increase in water consumption was maintained during the withdrawal phase for male rats in Group 3 (4500 ppm) and 4 (9000 ppm).

No treatment-related findings were evident in blood biochemical parameters. Slight difference in red cell parameters (reduced haemoglobin, packed cell volume; red blood cell count and increased mean corpuscular volume and mean corpuscular haemoglobin) seen in blood samples measured at the end of the period of exposures in Groups 3 (4500 ppm) and 4 (9000 ppm) compared with control data may be treatment-related. This difference was still present in withdrawal rats. In the absence of any bone marrow changes the above differences are equivocal. No treatment-related effects on bone myelograms were evident.

Reduced pH values in the urine of all male exposed rats and female Group 3 (4500 ppm) at the end of exposure may indicate the presence of an acidic metabolite. Following the withdrawal period pH values for male rats exposed to HFC 143 were similar to those for control rats and pH values for female rats exposed to HFC 143 were only slightly lower than control values. This indicates that any effect on urinary pH as a result of exposure to HFC 143 was reversible.

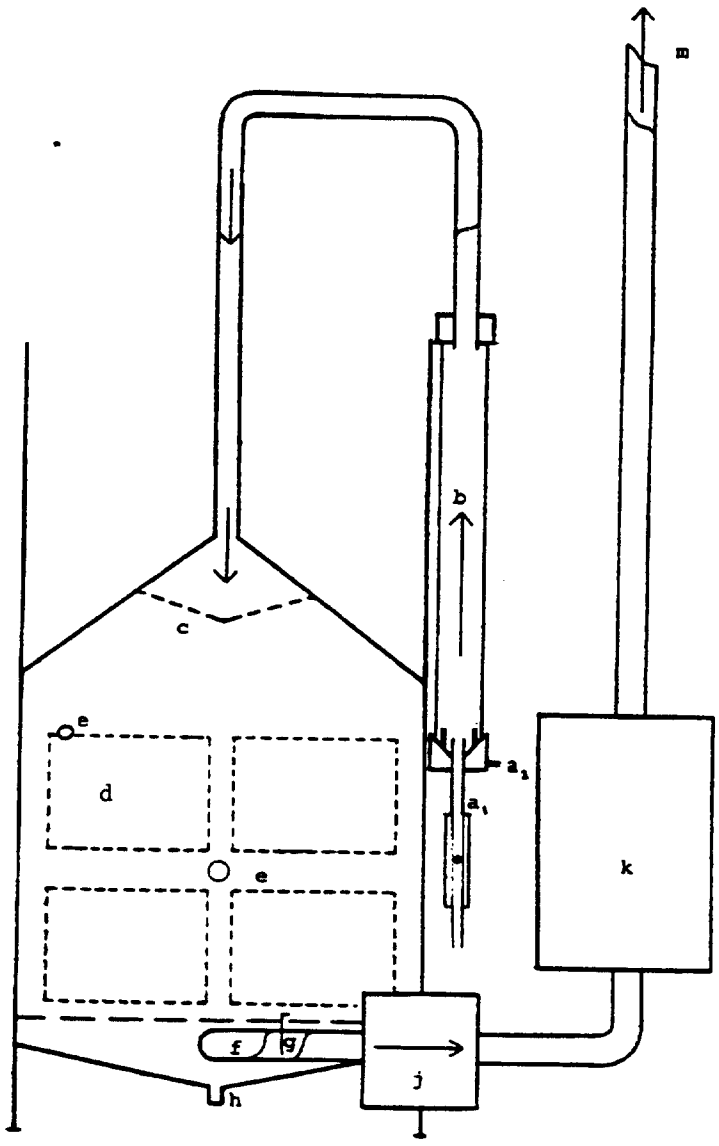
Urinary fluoride levels for all exposed groups were higher than control at the end of the exposure period. The increased levels of fluoride were probably due to excretion of inorganic fluoride derived from the test substance. Following a 4-week withdrawal period fluoride levels for animals exposed at 4500 ppm or higher remained higher than control values but were reduced compared with values obtained at the end of the exposure period. This observation is consistent with clearance of the metabolic breakdown products of HFC 143 from the body following cessation of exposure.

A dose-related reduction in the size of testes of male rats in all exposed groups, seen at necropsy, was confirmed by a similar dose-related reduction in the weight of the epididymides and testes. These findings were also recorded for the withdrawal rats in Group 2 (2250 ppm), 3 (4500 ppm) and 4 (9000 ppm).

Histological examination of the testes and epididymides revealed degeneration of the seminiferous epithelium, reduction/absence of spermatozoa and the presence of degenerate round germ cell (in the epididymides). The degenerate changes were present in terminal and withdrawal kill rats, indicating little or no recovery.

FIGURE 1

Exposure system

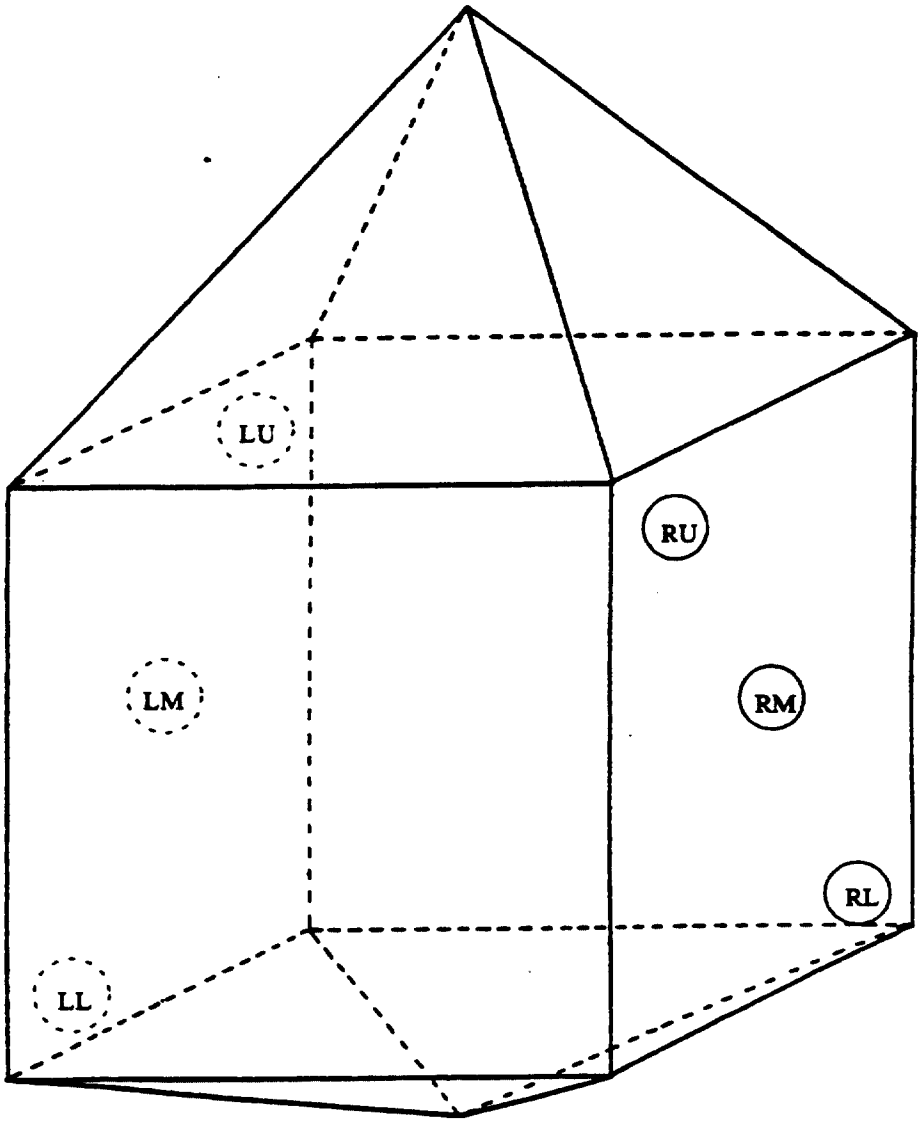


KEY

a_1	Vapour inlet	f	Extract plenum
a_2	Diluent air	g	Gate valve controller
b	Elutriation column	h	Drain valve
c	Perforate baffle	j	Pre-filter
d	Animal exposure cage	k	Powered extract/filter unit
e	Sample ports	m	Extract manifold

FIGURE 2

Location of sample ports on exposure chambers



KEY

LU	Left upper	RU	Right upper
LM	Left middle	RM	Right middle
LL	Left lower	RL	Right lower

FIGURE 3

Bodyweights - group mean values

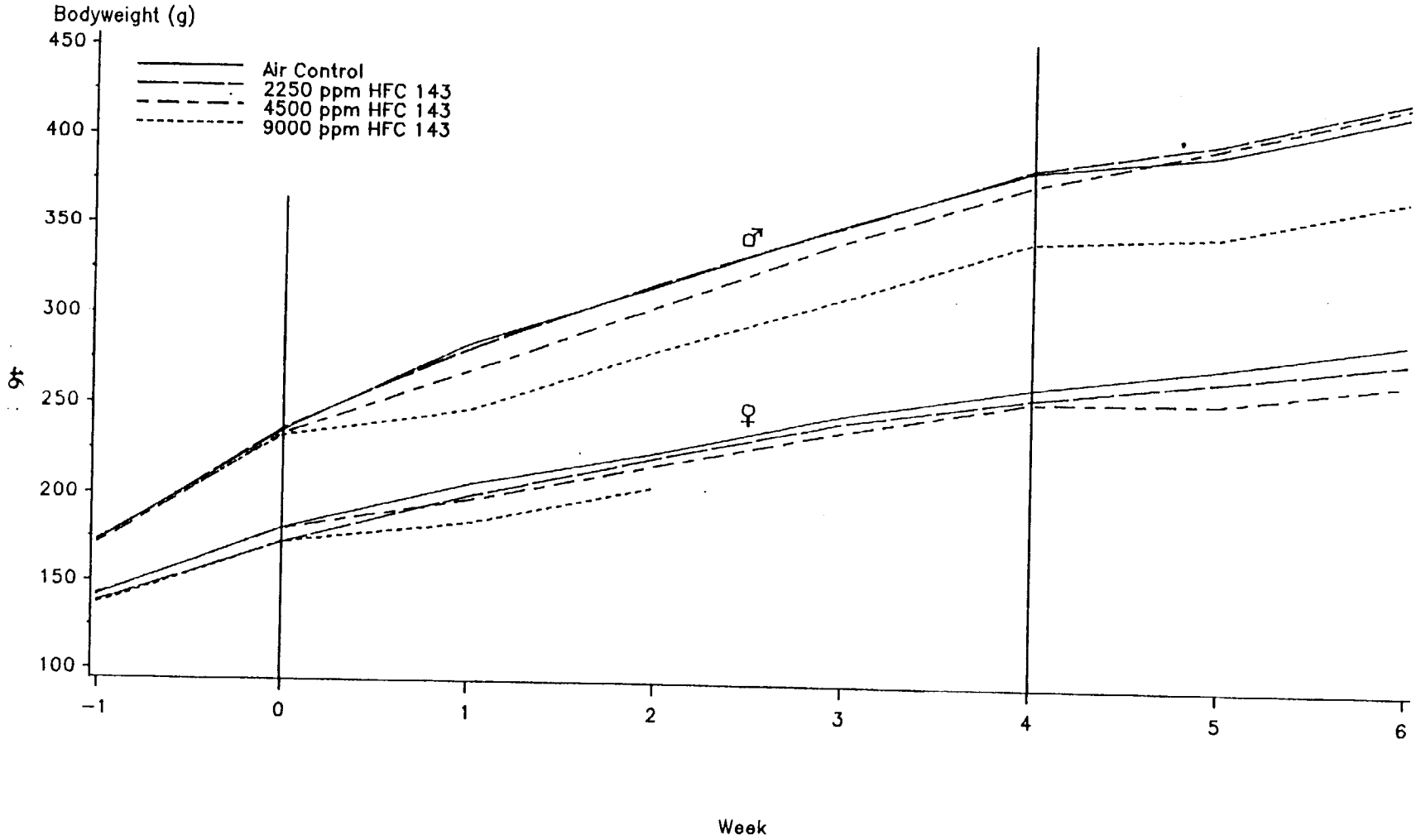


TABLE 1

Chamber atmosphere analysed concentration of HFC 143 - exposure mean values (ppm)

Exposure	Group		
	2 Low dose HFC 143 2250 ppm	3 Inter dose HFC 143 4500 ppm	4 High dose HFC 143 9000 ppm
1	2234	4461	8935
2	2256	4438	8914
3	2222	4413	8970
4	2218	4477	8978
5	2259	4550	9089
6	2219	4487	8985
7	2198	4173	8953
8	2222	4556	8950
9	2198	4491	9013
10	2197	4274	8844
11	2157	4249	8683
12	2249	4484	9006
13	2260	4540	8884
14	2247	4469	8875
15	2213	4450	8883
16	2198	4324	8875
17	2250	4478	8954
18	2262	4522	8899
19	2239	4529	8950
20	2218	4467	8859
21	2232	4465	8828
Study mean	2226	4443	8920
SD	27.0	102.9	83.8

SD Standard deviation

TABLE 2

Chamber temperature and relative humidity - exposure mean values

Exposure	Group							
	1 (Air Control)		2 (Low dose HFC 143)		3 (Inter dose HFC 143)		4 (High dose HFC 143)	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
1	23.4	34	23.6	40	22.5	24	23.0	29
2	23.6	26	23.8	37	22.3	25	22.4	30
3	23.9	28	23.9	39	22.8	23	22.2	31
4	23.2	27	23.2	41	21.8	28	21.7	30
5	23.3	30	23.3	42	22.1	27	21.9	32
6	23.3	31	23.3	40	22.3	25	21.7	33
7	22.9	31	23.2	41	22.2	25	21.4	32
8	23.0	26	22.7	41	21.8	24	21.2	31
9	22.8	30	22.8	43	21.7	25	21.3	29
10	23.7	35	23.7	45	22.7	28	22.3	36
11	23.8	30	23.5	48	22.7	26	22.4	29
12	23.7	32	23.8	43	22.8	28	22.3	31
13	23.8	30	23.4	43	22.8	27	22.2	32
14	23.5	32	23.9	41	22.8	26	22.3	30
15	23.6	33	23.7	42	22.6	26	21.7	28
16	23.0	32	23.4	42	22.3	25	21.2	26
17	22.7	30	22.7	40	21.7	24	20.4	25
18	23.8	32	24.0	41	22.9	27	21.9	30
19	23.7	34	23.8	43	22.7	27	21.8	29
20	23.9	34	24.0	45	23.2	29	21.9	31
21	23.7	35	23.8	44	22.7	20	21.8	30
Study mean	23.4	31	23.5	42	22.4	26	21.9	30

TABLE 3

Chamber distribution of HFC 143 vapour (ppm)

Exposure 2 (17 December 1993)

Sampling part[a]	Group		
	2 Low dose HFC 143	3 Inter dose HFC 143	4 High dose HFC 143
Lower	2228	4441	8837
Middle	2264	4284	8849
Upper	2291	4586	8934
Mean	2261	4437	8873
Variation*	2.8	6.8	1.1

Exposure 20 (12 January 1994)

Sampling part[a]	Group		
	2 Low dose HFC 143	3 Inter dose HFC 143	4 High dose HFC 143
Lower	2215	4465	8451
Middle	2174	4381	8970
Upper	2210	4513	8680
Mean	2200	4453	8700
Variation*	1.9	3.0	6.0

$$* \text{ variation} = \left[\frac{\text{Range} \times 100}{\text{Mean}}\right]$$

[a] See Figure 2

TABLE 4

Clinical signs - during exposure

Group	Observation	Exposure																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	NAD	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	NAD		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Convulsion[a]	✓																				
	Prone with slow breathing[a]	✓																				
3	NAD	✓							✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓
	Hunched posture		✓	✓										✓								
	Eyes shut or half-closed		✓	✓	✓	✓	✓	✓		✓				✓								
	Piloerection		✓	✓		✓		✓														
	Exaggerated breathing movement		✓																			
4	NAD																				✓	
	Convulsion[a]	✓																				
	Prone with slow breathing[a]	✓																				
	Hyperactive[b]		✓											✓								
	"Popping"[c]		✓						✓													
	Hunched posture		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓			✓
	Abnormal breathing movement[d]		✓		✓				✓													
	Eyes shut or half-closed		✓	✓	✓	✓	✓	✓	✓	✓				✓	✓	✓		✓	✓	✓		✓
	Piloerection		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		✓		

NAD Nothing abnormal detected
a Observation applies to single rat
b Observation describes hyperactivity in preening and restlessness
c Observation describes vertical jumping
d Observation described pronounced slow, rapid and exaggerated breathing

TABLE 5

Bodyweights - group mean values (g)

Week	Group and dosage (ppm)							
	1♂ Air Control	2♂ 2250 ppm HFC143	3♂ 4500 ppm HFC143	4♂ 9000 ppm HFC143	1♀ Air Control	2♀ 2250 ppm HFC143	3♀ 4500 ppm HFC143	4♀ 9000 ppm HFC143
-1	172	173	171	173	142	138	142	137
0	235	237	234	233	181	173	181	173
1	284	281	269	247	207	200	198	185
2	317	319	306	281	225	222	218	206
3	353	352	343	312	247	243	238	(a)
4	384	385	376	344	263	257	255	
Withdrawal								
4	380	385	377	333	267	262	250	
5	394	401	399	349	275	268	256	
6	418	426	423	370	290	280	268	
Gain								
-1 to 0	63	64	63	60	39	35	39	36
0 to 4	149	149	143	111**	82	85	75	
4 to 6	38	41	46	38	23	18	18	

** P <0.01 compared with control gain using Williams' test

(a) All female rats in Group 4 dead Week 3 of study

TABLE 6

Food consumption - group mean values (g)

Measured twice a week (Monday & Thursday)

Week	Group and dosage (ppm)							
	1♂ Air Control	2♂ 2250 ppm HFC143	3♂ 4500 ppm HFC143	4♂ 9000 ppm HFC143	1♀ Air Control	2♀ 2250 ppm HFC143	3♀ 4500 ppm HFC143	4♀ 9000 ppm HFC143
-0.4	81	83	81	82	62	63	61	60
-0.1	30	31	30	29	22	22	23	21
0.4	123	115	96	63	90	84	66	34
1	95	89	86	68	72	65	62	48
1.4	122	121	119	121	89	87	86	89
2	65	64	60	52	46	48	43	41
2.4	119	124	120	118	95	92	94	94
3	67	66	64	56	48	51	47	19
3.4	123	127	125	123	99	96	95	(a)
4	88	89	82	82	67	67	60	
Withdrawal								
4.4	119	121	126	108	93	88	93	
5	100	102	106	100	85	76	71	
5.4	133	127	135	123	109	95	97	
6	93	92	91	84	78	69	68	
Cumulative (weeks)								
1 to 4	802	794	752	694**	605	589	552*	
4 to 6	446	442	458	414	365	328	329	

* P <0.01, ** P <0.05 compared with control cumulative data using Williams' test

(a) All female rats in Group 4 dead Week 3 of study

TABLE 7

Water consumption - group mean values (g)

Week	Group and dosage (ppm)							
	1♂ Air Control	2♂ 2250 ppm HFC143	3♂ 4500 ppm HFC143	4♂ 9000 ppm HFC143	1♀ Air Control	2♀ 2250 ppm HFC143	3♀ 4500 ppm HFC143	4♀ 9000 ppm HFC143
-1	229.6	211.6	219.0	220.0	188.7	179.1	194.2	186.5
1	229.6	207.5	246.4	221.8	177.5	166.6	225.6	147.4
2	209.2	191.8	219.0	232.5	157.1	153.4	177.3	215.6
3	213.6	197.6	207.1	220.3	173.8	174.6	214.4	207.3
4	220.3	207.2	229.9	235.8	176.8	173.0	212.5	(a)
Withdrawal								
5	222.1	205.1	248.9	255.3	192.7	186.7	192.8	
6	242.0	209.8	257.2	268.0	212.2	244.8	204.2	
Cumulative (Weeks)								
1 to 4	873	804	902	932	685	668	830	
	(2.937)	(2.904)	(2.945)	(2.961)	[679]	[671]	[778]*	
4 to 6	464	415	506	523	405	432	397	

* P <0.05 compared with control data using Williams' test

( ) Analysis performed using log transformation. Log mean values in parentheses

[ ] Analysis using distribution-free method. Median values in square brackets

(a) All female rats in Group 4 dead Week 3 of study

TABLE 8

Haematology - group mean values

Week 5 (at the end of the period of exposures)

Group	PCV	Hb	RBC	MCHC	MCV	MCH	Retic	WBC + Diff $\times 10^3/mm^3$						Plts	TT
	%	g/dl	$\times 10^6/mm^3$	%	fl	pg	%	Total	N	L	E	B	M	$\times 10^3/mm^3$	s
1♂ Air Control	53	15.9	7.3	30.3	72	21.8	1.4	6.8	1.55	5.20	0.03	0.00	0.00	# 844 (810)	24
2♂ 2250 ppm HFC 143	51	15.7	7.0	30.7	73	22.3	0.8	7.3	2.66	4.63	0.03	0.00	0.03	839 (885)	24
3♂ 4500 ppm HFC 143	* 50	* 15.3	** 6.7	30.9	** 75	* 23.1	0.5	6.1	1.28	4.71	0.07	0.00	0.00	740 (884)	24
4♂ 9000 ppm HFC 143	* 50	* 15.4	** 6.7	30.7	** 75	* 23.1	1.7	8.2	2.13	6.06	0.02	0.00	0.02	831 (845)	22
1♀ Air Control	49	15.1	6.6	30.7	74	22.7	1.6	8.8	2.06	6.60	0.09	0.00	0.03	905	20
2♀ 2250 ppm HFC 143	50	15.0	6.8	30.3	73	22.1	1.7	6.7	1.39	5.20	0.06	0.00	0.03	883	21
3♀ 4500 ppm HFC 143	48	* 14.6	6.4	30.5	75	22.8	0.9	8.5	2.45	5.92	0.09	0.00	0.02	1053	20

* $P < 0.05$, ** $P < 0.01$ compared with control data using Williams' test

Distribution - free method of analysis applied to the data. Median values in parentheses

TABLE 9

Haematology - group mean values

Week 7 (at the end of the withdrawal period)

Group	PCV %	Hb g/dl	RBC $\times 10^6$/ mm^3	MCHC %	MCV fl	MCH pg	Retic %
1♂ Air Control	50	15.4	7.4	31.0	68	20.9	2.3
2♂ 2250 ppm HFC 143	49	15.6	7.0	31.7	** 71	** 22.4	1.8
3♂ 4500 ppm HFC 143	51	16.2	7.3	31.4	** 71	** 22.3	2.5
4♂ 9000 ppm HFC 143	48	15.0	** 6.8	31.2	** 71	** 22.2	2.8
1♀ Air Control	51	15.8	7.2	30.8	71	21.9	2.2
2♀ 2250 ppm HFC 143	52	15.9	7.2	30.7	72	22.1	1.8
3♀ 4500 ppm HFC 143	50	15.5	6.8	31.3	* 73	** 22.9	2.0

* P <0.05, ** P <0.01 compared with control data using Williams' test

TABLE 10

Biochemistry - group mean values

Week 5 (at the end of the period of exposures)

Group	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml
		Total	Alb	Glob					
1♂ Air Control	111	6.5	2.9	3.5	14	0.5	337	32	57
2♂ 2250 ppm HFC 143	116	6.3	2.9	3.4	13	0.5	347	31	55
3♂ 4500 ppm HFC 143	114	6.1	2.9	3.2	14	0.6	299	28	54
4♂ 9000 ppm HFC 143	113	6.3	2.9	3.4	13	0.5	307	29	62
1♀ Air Control	113	6.7	3.1	3.6	17	0.6	232	26	50
2♀ 2250 ppm HFC 143	115	6.6	3.1	3.5	17	0.6	198	26	56
3♀ 4500 ppm HFC 143	119	6.4	3.1	3.3	16	0.5	219	24	47

TABLE 10

(Biochemistry - continued)

Week 5 (at the end of the period of exposures)

Group	γGT mU/ ml	CPK mU/ ml	Bili- rubin mg/dl	Na mEq/ l	K mEq/ l	Ca mEq/ l	P mEq/ l	Cl mEq/ l	Chol mg/dl
1♂ Air Control	<1	187	0.1	143	3.2	5.2	4.3	98	68
2♂ 2250 ppm HFC 143	<1	122	0.1	144	3.3	5.2	4.2	100	69
3♂ 4500 ppm HFC 143	<1	116	0.1	144	3.2	5.1	4.5	100	56
4♂ 9000 ppm HFC 143	<1	114	0.1	145	3.3	5.3	4.6	100	87
1♀ Air Control	<1	133	0.1	142	3.6	5.3	4.1	100	78
2♀ 2250 ppm HFC 143	<1	150	0.1	142	3.2**	5.4	3.9	99	69
3♀ 4500 ppm HFC 143	<1	116	0.2	142	3.1**	5.2	4.0	99	73

** P <0.01 compared with control data using Williams' test

TABLE 11

Urinalysis - group mean values

Week 5 (at the end of the period of exposures)

Group	Volume ml	pH	SG	Protein mg/dl
1♂ Air Control	6.8	6.8	1034	134
2♂ 2250 ppm HFC 143	5.5	** 6.5	1037	153
3♂ 4500 ppm HFC 143	5.4	** 6.3	1034	139
4♂ 9000 ppm HFC 143	5.4	** 6.2	1039	139
1♀ Air Control	4.2	6.5	1040	76
2♀ 2250 ppm HFC 143	4.2	6.4	1037	65
3♀ 4500 ppm HFC 143	5.5	** 6.0	1031	58

** P <0.01 compared with control data using Williams' test

TABLE 12

Urinalysis - group mean values

Week 7 (at the end of the withdrawal period)

Group	Vol-ume ml	pH
1♂ Air Control	8.6	6.6
2♂ 2250 ppm HFC 143	8.1	6.8
3♂ 4500 ppm HFC 143	7.3	6.6
4♂ 9000 ppm HFC 143	7.6	6.8
1♀ Air Control	5.5	6.7
2♀ 2250 ppm HFC 143	5.5	6.6
3♀ 4500 ppm HFC 143	4.1	6.4

TABLE 13

Urinary inorganic fluoride - group mean values

Week 5 (at the end of the period of exposures)

Group	Urine volume (ml)	Fluoride concentration (μg/ml)	Total fluoride (μg)
1♂ Air Control	7.94	1.69	12.53
2♂ 2250 ppm HFC 143	6.08	3.38**	20.00**
3♂ 4500 ppm HFC 143	5.64	4.17**	23.19**
4♂ 9000 ppm HFC 143	6.88	3.66**	23.70**
1♀ Air Control	4.94	1.70	7.78
2♀ 2250 ppm HFC 143	4.56	2.69**	11.77**
3♀ 4500 ppm HFC 143	4.84	3.49**	15.62**
4♀ 9000 ppm HFC 143	(3.6)	(4.88)	(14.79)

( ) Samples removed 5 January 1994, prior to sacrifice (Week 3). Not included in statistical analysis

** P <0.01 compared with control data using Williams' test

TABLE 14

Urinary inorganic fluoride - group mean values

Week 7 (at the end of the withdrawal period)

Group	Urine volume (ml)	Fluoride concentration (μg/ml)	Total fluoride (μg)
1♂ Air Control	8.6	1.46	12.10
2♂ 2250 ppm HFC 143	8.1	1.58	12.73
3♂ 4500 ppm HFC 143	7.3	2.24*	14.53
4♂ 9000 ppm HFC 143	7.6	2.25*	15.26*
1♀ Air Control	5.5	1.22	6.59
2♀ 2250 ppm HFC 143	5.5	1.48	8.09
3♀ 4500 ppm HFC 143	4.1	1.96**	7.81
4♀ 9000 ppm HFC 143	(All dead by Week 3 of study)		

* P <0.05, ** P <0.01 compared with control data using Williams' test

TABLE 15

Macroscopic findings - unscheduled deaths

Removal reason: Intercurrent	Group 4 Males	Group 4 Females
Animals on study	10	10
Animals completed	2	6
Fur		
Stained - perinasal region	1	1
Moist - perinasal region	0	1
Brain		
Congested	0	1
Lymph Nodes - Inguinal		
Congested	0	1
Thoracic Cavity		
Contained serous fluid	0	2
Contained serosanguineous fluid	1	1
Thymus		
Oedematous	1	2
Lungs		
Not collapsed	0	2
Firm	0	1
Congested	1	5
Haemorrhagic area	1	0
Haemorrhagic	1	0
Oedematous	0	2
Lymph Nodes - Tracheobronchial		
Not visible	0	1

TABLE 15

(Macroscopic findings - continued)

Removal reason: Intercurrent	Group 4 Males	Group 4 Females
Animals on study	10	10
Animals completed	2	6
Liver		
Pitted	0	1
Lobular markings accentuated	1	3
Stomach		
Contents watery	1	1
Empty	0	1
Stomach Corpus Mucosa		
Depressions	0	1
Stomach Antrum Mucosa		
White nodules, near to limiting ridge	1	1
Small Intestine		
Distended	0	1
Caecum		
Contents dark	1	0
Contents firm	0	1
Adrenals		
Congested	1	3

TABLE 16

Macroscopic findings - rats killed 5 January 1994 (Week 3)

Removal Reason: Terminal	Sex: Females	Group 4 9000 ppm
	Females on study Animals completed	10 4
Lymph Nodes - Cervical Enlarged		2
Thymus Congested		1
Liver Adhesions		1
Spleen A pale capsular area		1
Kidneys Pale cortical area/s Irregular cortical scarring Adhesions		1 1 1

TABLE 17

Macroscopic findings - termination of exposures

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3
	---------- Males ----------				---------- Females -----		
Animals on study	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5
Incisors							
Pale	0	0	0	0	0	1	0
Lymph Nodes - Cervical							
Enlarged	2	2	1	2	3	2	1
Lymph Nodes - Tracheobronchial							
Not visible	0	0	0	0	0	0	1
Liver							
Pale subcapsular area/s - median cleft	0	1	0	0	0	1	0
Necrotic lobe	0	0	1	0	0	0	0
Small lobe	0	0	1	0	0	0	0
Yellow lobe	0	0	1	0	0	0	0
Pancreas							
Congested	1	0	0	0	0	0	0
Stomach Corpus Mucosa							
Haemorrhagic depression	0	0	2	0	0	0	0
Stomach Antrum Mucosa							
Haemorrhagic depression	0	0	0	1	0	0	0
Kidneys							
Irregular cortical scarring	0	1	0	0	0	0	0

TABLE 17

(Macroscopic findings - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3
	Males				Females		
Animals on study Animals completed	10 5	10 5	10 5	10 4	10 5	10 5	10 5
Seminal Vesicles Contents minimal	0	0	1	0	0	0	0
Testes Small	0	2	3	4	0	0	0
Epididymides Small	0	1	2	4	0	0	0

TABLE 18

Macroscopic findings - at the end of the withdrawal period

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	---------- Males ----------				---------- Females ----------			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	0*
Fur								
Stained	0	1	0	0	0	0	0	0
Lymph Nodes - Cervical								
Enlarged	5	5	5	4	5	5	5	0
Congested	0	1	0	0	0	0	0	0
Lymph Nodes - Deep Cervical								
Enlarged	1	0	0	0	0	0	0	0
Lungs								
Adhesions	0	0	1	0	0	0	0	0
Liver								
Pale subcapsular area/s - median cleft	3	1	2	1	2	4	2	0
Lobular markings accentuated	1	1	1	0	0	0	0	0
Stomach Corpus Mucosa								
Haemorrhagic depression	0	0	1	0	0	0	0	0
Testes								
Small	0	5	5	4	0	0	0	0
Epididymides								
Small	0	5	5	4	0	0	0	0
Uterus								
Fluid distension	0	0	0	0	2	1	2	0

* All dead Week 3 of study

TABLE 19

Bone myelograms - group mean values

Week 5 (at the end of the period of exposures)

Group	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♂ Air Control	37.7	28.4	33.9	1.45
2♂ 2250 ppm HFC 143	42.0	22.5	35.5	1.96
3♂ 4500 ppm HFC 143	35.7	19.8	44.5	1.82
4♂ 9000 ppm HFC 143	35.1	26.9	38.0	1.52
1♀ Air Control	35.7	24.0	40.3	1.54
2♀ 2250 ppm HFC 143	38.8	28.3	32.9	1.39
3♀ 4500 ppm HFC 143	29.3	26.6	44.1	1.14
4♀ 9000 ppm* HFC 143				

* All dead by Week 3 of study

TABLE 20

Bone myelograms - group mean values

Week 7 (at the end of the withdrawal period)

Group	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♂ Air Control	43.7	27.1	29.2	1.66
2♂ 2250 ppm HFC 143	42.0	22.7	35.3	1.97
3♂ 4500 ppm HFC 143	40.1	26.0	33.9	1.61
4♂ 9000 ppm HFC 143	42.5	29.6	27.9	1.45
1♀ Air Control	40.6	27.5	31.9	1.53
2♀ 2250 ppm HFC 143	38.0	28.8	33.2	1.41
3♀ 4500 ppm HFC 143	40.7	28.0	31.3	1.47

TABLE 21

Organ weights - group mean values

Week 5 (At the end of the period of exposures)

Group	Body wt* g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Epididymides L g	Epididymides R g
1♂ Air Control	350	A 1.30 (1.28)	A 13.1 (12.8)	A 2.51 (2.47)	49.7	A 1.54 (1.504)	A 1.57 (1.544)	0.438	A 0.435 (0.429)
2♂ 2250 ppm HFC143	353	1.34 (1.31)	* 11.3 (10.8)	2.43 (2.37)	52.4	** 1.05 (1.002)	** 1.01 (0.962)	* 0.352	* 0.369 (0.360)
3♂ 4500 ppm HFC143	343	1.23 (1.23)	* 10.5 (10.5)	2.38 (2.38)	53.0	** 0.80 (0.804)	** 0.81 (0.813)	* 0.353	** 0.336 (0.336)
4♂ 9000 ppm HFC143	322	1.29 (1.34)	* 10.4 (11.4)	2.30 (2.42)	55.8	** 0.63 (0.727)	** 0.61 (0.703)	** 0.269	** 0.261 (0.279)

* P <0.05, ** P <0.01 compared with control data using Williams' test

A Statistical analysis performed using bodyweight as covariate. Adjusted mean values in parentheses

TABLE 21

(Organ weights - continued)

Week 5 (at the end of the period of exposures)

Group	Body wt	Lungs	Liver	Kidneys	Adrenals
	g	g	g	g	mg
1♀ Air Control	228	1.09	A 7.4 (7.5)	A 1.59 (1.609)	A 64.4 (64.8)
2♀ 2250 ppm HFC 143	228	1.05	8.2 (8.3)	1.74 (1.75)	64.5 (64.9)
3♀ 4500 ppm HFC 143	234	1.07	* 8.6 (8.4)	1.81 (1.79)	66.7 (65.8)

* $P < 0.05$ compared with control data using Williams' test

A Statistical analysis performed using bodyweight as covariate. Adjusted mean values in parentheses

TABLE 22

Organ weights - group mean values

Week 7 (at the end of the withdrawal period)

Group	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Epididymides L g	Epididymides R g
1♂ Air Control	383	A 1.37 (1.36)	A 10.3 (10.2)	A 2.35 (2.34)	52.6	1.58	1.60	0.504	0.510
2♂ 2250 ppm HFC 143	390	1.50 (1.49)	10.5 (10.2)	2.41 (2.36)	58.9	** 0.98	** 0.96	** 0.391	** 0.391
3♂ 4500 ppm HFC 143	397	1.35 (1.33)	11.3 (10.7)	2.35 (2.27)	62.1	** 0.63	** 0.66	** 0.300	** 0.305
4♂ 9000 ppm HFC 143	340	1.35 (1.40)	8.9 (10.2)	2.20 (2.36)	61.3	** 0.62	** 0.59	** 0.268	** 0.293

** P <0.01 compared with control data using Williams' test

A Statistical analysis performed using bodyweight as covariate. Adjusted mean values in parentheses

TABLE 22

(Organ weights - continued)

Week 7 (at the end of the withdrawal period)

Group	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg
1♀ Air Control	269	A 1.14 (1.12)	A 7.9 (7.7)	A 1.65 (1.62)	A 69.1 (68)
2♀ 2250 ppm HFC 143	269	1.18 (1.17)	* 9.1 (8.9)	1.76 (1.73)	72.1 (71)
3♀ 4500 ppm HFC 143	256	1.22 (1.25)	* 8.1 (8.4)	1.66 (1.72)	69.8 (72)

* P <0.05 compared with control gain using Williams' test

A Statistical analysis performed using bodyweight as covariate. Adjusted mean values in parentheses

TABLE 23

Microscopic pathology incidence summary - unscheduled deaths

Removal reason: Intercurrent	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	0	0	0	2	0	0	0	6
Lungs								
Examined	0	0	0	2	0	0	0	6
Pneumonia (Total)	0	0	0	0	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Increased numbers of alveolar macrophages (Total)	0	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	0	2
Vascular congestion	0	0	0	1	0	0	0	5
Perivascular oedema	0	0	0	1	0	0	0	6
Intra-alveolar haemorrhage	0	0	0	1	0	0	0	3
Intra-alveolar oedema	0	0	0	1	0	0	0	1
Thymus								
Examined	0	0	0	1	0	0	0	2
Involution (Total)	0	0	0	1	0	0	0	2
Moderate	0	0	0	1	0	0	0	2
Vascular congestion	0	0	0	1	0	0	0	0
Liver								
Examined	0	0	0	1	0	0	0	3
Centrilobular fibrosis (Total)	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Centrilobular hepatocyte necrosis (Total)	0	0	0	0	0	0	0	3
Minimal	0	0	0	0	0	0	0	3
Adrenals								
Examined	0	0	0	1	0	0	0	3
No abnormalities detected	0	0	0	1	0	0	0	2
Sinusoidal congestion	0	0	0	0	0	0	0	1
Stomach								
Examined	0	0	0	2	0	0	0	2
No abnormalities detected	0	0	0	1	0	0	0	0

TABLE 23

(Microscopic pathology incidence summary - continued)

Removal reason: Intercurrent	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
	---------- Males ----------				---------- Females ----------			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	0	0	0	2	0	0	0	6
Stomach	(Continued)							
Erosion of glandular epithelium	0	0	0	0	0	0	0	1
Focus of ectopic non-glandular epithelium within the glandular mucosa	0	0	0	1	0	0	0	1
Lymph Nodes - Inguinal								
Missing	0	0	0	0	0	0	0	1
Duodenum								
Examined	0	0	0	0	0	0	0	1
No abnormalities detected	0	0	0	0	0	0	0	1
Jejunum								
Examined	0	0	0	0	0	0	0	1
No abnormalities detected	0	0	0	0	0	0	0	1
Caecum								
Examined	0	0	0	1	0	0	0	1
No abnormalities detected	0	0	0	0	0	0	0	1
Vascular congestion (Total)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Brain								
Examined	0	0	0	0	0	0	0	1
Vascular congestion	0	0	0	0	0	0	0	1
Factors Contributory To Death								
Examined	0	0	0	2	0	0	0	6
Haemorrhage in the lung	0	0	0	1	0	0	0	2
Vascular congestion and oedema in the lung	0	0	0	1	0	0	0	4

TABLE 24

Microscopic pathology incidence summary - termination of exposures

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4*
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	4
Nasal Turbinates								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	4	5	0	0	4
Larynx								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	4	5	0	0	4
Trachea								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	4	5	0	0	4
Tracheal Bifrucation								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	4	5	0	0	4
Lungs								
Examined	5	5	5	4	5	5	5	4
No abnormalities detected	3	3	5	3	2	5	5	4
Pneumonitis (Total)	2	1	0	1	2	0	0	0
Minimal	2	1	0	1	2	0	0	0
Vascular congestion	0	0	0	0	1	0	0	0
Intra-alveolar haemorrhage	0	1	0	0	0	0	0	0
Thymus								
Examined	0	0	0	0	0	0	0	1
Vascular congestion	0	0	0	0	0	0	0	1
Heart								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	3	0	0	3	5	0	0	4
Myocardial fibrosis (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0

* Sacrificed during Week 3 of the study

TABLE 24

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4*
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	4
Heart	(Continued)							
Myocardial inflammation (Total)	1	0	0	1	0	0	0	0
Minimal	1	0	0	1	0	0	0	0
Lymph Nodes - Cervical								
Examined	2	0	0	2	3	0	0	2
Lymphoid proliferation (Total)	2	0	0	2	3	0	0	2
Minimal	2	0	0	2	2	0	0	2
Moderate	0	0	0	0	1	0	0	0
Liver								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	1	0	0	1	2	0	0	1
Bile duct hyperplasia (Total)	0	0	0	0	1	0	0	0
Trace	0	0	0	0	1	0	0	0
Extramedullary haemopoiesis (Total)	4	0	0	3	2	0	0	2
Minimal	4	0	0	3	2	0	0	2
Adhesion	0	0	0	0	0	0	0	1
Spleen								
Examined	0	0	0	0	0	0	0	1
Capsular thickening (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Pancreas								
Examined	1	0	0	0	0	0	0	0
Vascular congestion	1	0	0	0	0	0	0	0
Kidneys								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	3	4	0	0	1
Pyelitis (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1

* Sacrificed during Week 3 of the study

TABLE 24

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3 *	Group 4*
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	4
Kidneys	(Continued)							
Cortical scarring (Total)	0	0	0	0	0	0	0	2
Minimal	0	0	0	0	0	0	0	2
Mineral casts in tubules at the corticomedullary junction (Total)	0	0	0	1	0	0	0	2
Minimal	0	0	0	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Basophilic cortical tubules (Total)	0	0	0	1	1	0	0	0
Minimal	0	0	0	1	1	0	0	0
Urothelial hyperplasia in the pelvis (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Cortical collecting ducts lined by clear cells (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Epididymides								
Examined	5	5	5	4	0	0	0	0
No abnormalities detected	5	0	0	0	0	0	0	0
Spermatozoa absent from caput	0	5	5	4	0	0	0	0
Spermatozoa absent from cauda	0	1	3	3	0	0	0	0
Reduced numbers of spermatozoa in cauda (Total)	0	2	2	1	0	0	0	0
Moderate	0	2	1	0	0	0	0	0
Marked	0	0	1	1	0	0	0	0
Degenerate round germ cells (Total)	0	5	5	4	0	0	0	0
Minimal	0	4	2	2	0	0	0	0
Moderate	0	1	3	2	0	0	0	0
Testes								
Examined	5	5	5	4	0	0	0	0
No abnormalities detected	5	0	0	0	0	0	0	0

* Sacrificed during Week 3 of the study

TABLE 24

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4*
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	4
Testes	(Continued)							
Atrophic tubules lined only by Sertoli cells (Total)	0	2	3	4	0	0	0	0
Minimal	0	2	1	0	0	0	0	0
Moderate	0	0	1	4	0	0	0	0
Marked	0	0	1	0	0	0	0	0
Reduction/absence of tailed spermatids (Total)	0	5	5	4	0	0	0	0
Minimal	0	3	0	0	0	0	0	0
Moderate	0	0	1	0	0	0	0	0
Marked	0	2	4	4	0	0	0	0
Reduction/absence of round spermatids (Total)	0	5	5	4	0	0	0	0
Minimal	0	3	2	0	0	0	0	0
Moderate	0	2	3	0	0	0	0	0
Marked	0	0	0	4	0	0	0	0
Reduction/absence of spermatocytes (Total)	0	5	5	4	0	0	0	0
Trace	0	1	0	0	0	0	0	0
Minimal	0	4	2	0	0	0	0	0
Moderate	0	0	3	2	0	0	0	0
Marked	0	0	0	2	0	0	0	0
Reduction/absence of spermatogonia (Total)	0	0	1	4	0	0	0	0
Minimal	0	0	1	3	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Vacuoles in seminiferous epithelium (Total)	0	5	5	4	0	0	0	0
Minimal	0	5	3	4	0	0	0	0
Moderate	0	0	2	0	0	0	0	0
Multinucleate round spermatids (Total)	0	2	3	4	0	0	0	0
Minimal	0	2	3	4	0	0	0	0

* Sacrificed during Week 3 of the study

TABLE 24

(Microscopic pathology incidence summary - continued)

Removal reason: Terminal	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4*
	Males				Females			
Animals on study	10	10	10	10	10	10	10	10
Animals completed	5	5	5	4	5	5	5	4
Testes	(Continued)							
Multinucleate spermatocytes (Total)	0	0	0	2	0	0	0	0
Trace	0	0	0	2	0	0	0	0
Degenerate germ cells mainly spermatocytes (Total)	0	5	5	4	0	0	0	0
Minimal	0	4	5	2	0	0	0	0
Moderate	0	1	0	2	0	0	0	0
Adrenals								
Examined	5	0	0	4	5	0	0	4
No abnormalities detected	5	0	0	4	5	0	0	4
Stomach								
Examined	0	0	0	1	0	0	0	0
No abnormalities detected	0	0	0	1	0	0	0	0

* Sacrificed during Week 3 of the study

TABLE 25

Microscopic pathology incidence summary - at the end of the withdrawal period

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4
	---------- Males ----------			
Animals on study	10	10	10	10
Animals completed	5	5	5	4
Epididymides				
Examined	5	5	5	4
No abnormalities detected	5	0	0	0
Spermatozoa absent from caput	0	5	5	4
Spermatozoa absent from cauda	0	2	1	4
Reduced numbers of spermatozoa in cauda (Total)	0	3	4	0
Moderate	0	2	0	0
Marked	0	1	4	0
Degenerate round germ cells (Total)	0	5	5	4
Minimal	0	5	1	2
Moderate	0	0	4	2
Testes				
Examined	5	5	5	4
No abnormalities detected	5	0	0	0
Atrophic tubules lined only by Sertoli cells (Total)	0	5	5	4
Minimal	0	4	2	2
Moderate	0	0	1	0
Marked	0	1	2	2
Reduction/absence of tailed spermatids (Total)	0	4	5	3
Minimal	0	3	2	0
Moderate	0	1	0	0
Marked	0	0	3	3
Reduction/absence of round spermatids (Total)	0	2	5	3
Trace	0	1	0	0
Minimal	0	1	2	0
Moderate	0	0	2	2
Marked	0	0	1	1

TABLE 25

(Microscopic pathology incidence summary - continued)

Removal reason: Recovery	Group 1	Group 2	Group 3	Group 4
	---------- Males ----------			
Animals on study	10	10	10	10
Animals completed	5	5	5	4
Testes	(Continued)			
Reduction/absence of spermatocytes (Total)	0	2	5	3
Trace	0	0	1	0
Minimal	0	2	3	3
Moderate	0	0	1	0
Reduction/absence of spermatogonia (Total)	0	1	2	0
Minimal	0	1	2	0
Vacuoles in seminiferous epithelium (Total)	0	5	5	3
Minimal	0	4	5	1
Moderate	0	1	0	2
Multinucleate round spermatids (Total)	0	0	1	1
Trace	0	0	1	1
Degenerate germ cells mainly spermatocytes (Total)	0	4	3	3
Trace	0	3	2	2
Minimal	0	1	1	1

APPENDIX 1

Special Diet Services Rat and Mouse Maintenance Diet

Composition and quality assurance aspects of rodent diet and water

SDS Rat and Mouse No. 1 SQC modified maintenance diet is a fixed formula diet. Each batch of diet is analysed for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess, may have an undesirable effect on the test system.

Prior to release of diet for use HRC Quality Assurance Department checks each certificate of analysis for conformity with the specification detailed below. Occasional slight deviations to this specification may be permitted.

Nutrients	Target level	Tolerance %	Acceptable range			
Moisture	10.0	+25			12.5	% max
Crude fat	3.0	±30	2.0	-	4.0	%
Crude protein	14.5	±15	12.0	-	16.5	%
Crude fibre	4.0	±50	2.0	-	6.0	%
Ash	5.0	±25	3.7	-	6.2	%
Calcium	0.9	±30	0.6	-	1.2	%
Phosphorus	0.6	±20	0.5	-	0.75	%
Sodium	0.25	±40	0.15	-	0.35	%
Chloride	0.5	±40	0.3	-	0.7	%
Potassium	0.9	±50	0.45	-	1.35	%
Magnesium	0.2	±50	0.1	-	0.3	%
Iron	200	±50	100	-	300	mg/kg
Copper	15	±60	6	-	24	mg/kg
Manganese	60	+60-40	36	-	100	mg/kg
Zinc	60	±50	30	-	90	mg/kg
Vitamin A	6	-50			3	iu/g min.
Vitamin E	70	-50			35	mg/kg min.

Contaminants	Maximum concentration	
Fluoride	20	mg/kg
Nitrate (as $NaNO_3$)	30	mg/kg
Nitrite (as $NaNO_2$)	10	mg/kg
Lead	2.0	mg/kg
Arsenic	1.0	mg/kg
Cadmium	0.7	mg/kg
Mercury	0.1	mg/kg
Selenium	0.6	mg/kg
Total Aflatoxins	5.0	mcg/kg
Total P.C.B.	50	mcg/kg
Total D.D.T.	250	mcg/kg
Dieldrin	50	mcg/kg
Lindane	300	mcg/kg
Heptachlor	20	mcg/kg
Malathion	5000	mcg/kg

APPENDIX 1

(Composition and quality assurance - continued)

Microbiological contents	Maximum concentration
Total viable organisms	25000 per g diet
Mesophilic spores	25000 per g diet
Salmonellae species	0 per g diet
Presumptive E. coli	0 per g diet
E. coli type 1	0 per g diet
Fungal units	300 per g diet
Antibiotic activity	0

APPENDIX 1

(Composition and quality assurance - continued)

The water supplied to HRC, by Anglian Water, is potable water for human consumption. Anglian Water takes its guidelines on water quality from the EEC directive relating to water for human consumption, viz: Council Directive 80/778/EEC.

Results of routine physical and chemical examination of drinking water at source as conducted, usually weekly by the supplier, are made available to HRC as quarterly summaries.

These results include levels of:

Nitrites	Potassium	Chloride
Nitrates	Silicon	Iron
Calcium	Arsenic	Selenium
Magnesium	Barium	Silver
Sodium	Antimony	Phosphorus

as well as concentrations of pesticides, related products, polycyclic aromatic hydrocarbons, haloforms, chlorophenols and polychlorinated biphenyls.

APPENDIX 2

Method of analysis for HFC 143 (trifluoroethane)

INSTRUMENTATION AND APPARATUS

Gas analysers:	2 Miran 1A-CVF infra-red gas analysers fitted with long pathlength cells. One monitoring the Low dose, the other monitoring the Intermediate and High doses.
Apparatus:	Glass gas-tight syringes and stoppered glass bottles.
	Tedlar® gas sample bag (SKC Inc Pa, USA.).

REAGENTS

HFC 143:	Supplied by Sponsor.

ANALYSER CONDITIONS

	Group 2 (Low dose)	Groups 3 (Intermediate dose and 4 (High dose)
Wavelength:	3.35 μm	4.85 μm
Pathlength:	3.75 m	5.25 m
Scale:	×1	×1
Range:	1 A	0.25 A
Slit width:	1 mm	0.5 mm
Meter response:	1	1
Calibration loop volume:	5.78 l	5.78 l

APPENDIX 2

(Method of analysis - continued)

CALIBRATION

A volume of HFC 143 gas was transferred from the pressurised cylinder into a Tedlar® gas sample bag. The bag was fitted with an injection port containing a self-sealing rubber septum.

A known volume of HFC 143 gas was withdrawn from the sample bag using a gas tight syringe, injected into each calibration loop of the gas analysers and the responses were recorded. Further volumes were injected and at each injection the cumulative absorbances were recorded. The results obtained are shown below.

Group 2 (Low dose)[a]		Groups 3 (Intermediate dose) and 4 (High dose)	
Equivalent concentration in loop* (ppm)	**Absorbance**	**Equivalent concentration in loop*** (ppm)	**Absorbance**
692	0.274	1730	0.152
1384	0.491	3460	0.301
2076	0.661	5190	0.447
2768	0.791	6920	0.589
3460	0.891	8651	0.730
		10381	0.869

[a] A power regression was used for Low dose concentration calculations to compensate for the curved standard response

* The calibration loops were each fitted with a sealed Tedlar® gas sample bag (SKC Inc, 334 Valley View Road, Eighty-four, PA15330, USA) to allow for volume occupied by HFC 143. The concentrations were calculated taking the total volumes into account

ANALYSIS OF SAMPLES

Chamber air samples were drawn into the sample loop by a vacuum pump. The analysers were connected to a chart recorder. Sampling and analysis were continuous throughout each 6-hour exposure for Group 2 (Low dose). For Groups 3 (Intermediate dose) and 4 (High dose) a system of valves allowed each chamber to be sampled alternately at 15-minute intervals.

APPENDIX 2

(Method of analysis - continued)

From the chart recording of absorption, each chamber concentration was determined at least 8 intervals during the exposure; using the standard response above, from the following expression:

$$C_x = \frac{A_x - I}{A_s}$$

Where C_x = concentration of HFC 143 in chamber (ppm)
A_x = absorbance of HFC 143 at the given wavelength
I = intercept of linear regression slope
A_s = response factor (area/unit concentration) for HFC 143

For Group 2, each chamber concentration was calculated from the standard responses above using the following expression:

$$l_n (Ax) = B^n l_n(C_x) + L_n I'$$

B = log of absorbance/log of concentration

APPENDIX 3

Analysed chamber concentrations of HFC 143 - individual values (ppm)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
1	2269	4550	8837
	2246	4429	9151
	2206	4489	8475
	2219	4296	9115
	2174	4381	8886
	2233	4429	9055
	2242	4550	9103
	2300	4429	8789
	2219	4598	9006
Mean	2234 (v=5.6)	4461 (v=6.8)	8935 (v=7.6)
2	2242	4417	8898
	2255	4586	8994
	2233	4320	8668
	2228	4441	8970
	2264	4586	9121
	2291	4441	8934
	2228	4284	8837
	2305	4453	8849
	2255	4417	8958
Mean	2256 (v=3.4)	4438 (v=6.8)	8914 (v=5.1)
3	2233	4429	8849
	2228	4525	9030
	2188	4429	8994
	2210	4320	8873
	2255	4453	9030
	2233	4344	9043
	2210	4381	8849
	2233	4441	9055
	2206	4393	9006
Mean	2222 (v=3.0)	4413 (v=4.6)	8970 (v=2.3)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
4	2094	4127	8837
	2179	4344	8898
	2242	4489	9067
	2251	4610	9115
	2233	4622	8898
	2242	4332	8716
	2206	4550	9272
	2273	4574	9018
	2246	4610	
Mean	2218 (v=8.1)	4473 (v=11.1)	8978 (v=6.2)
5	2233	4393	9357
	2282	4670	8741
	2219	4646	9477
	2323	4743	9018
	2264	4525	8849
	2242	4658	9260
	2260	4465	8898
	2269	4429	9115
	2242	4417	
Mean	2259 (v=4.6)	4550 (v=7.7)	9089 (v=6.8)
6	2143	4489	8680
	2161	4429	8777
	2260	4670	9115
	2219	4815	9332
	2260	4067	8922
	2255	4610	9018
	2224	4441	9175
	2215	4453	8910
	2237	4405	8934
Mean	2219 (v=5.3)	4487 (v=16.7)	8985 (v=7.3)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
7	2143	4610	8837
	2165	4417	8873
	2170	4381	8922
	2260	4320	9079
	2206	4670	8801
	2251	4477	9091
	2130	4248	8837
	2224	4646	9381
	2233	4489	8753
Mean	2198 (v=5.9)	4473 (v=9.4)	8953 (v=7.0)
8	2033	4574	8886
	2242	4465	9091
	2242	4586	8994
	2251	4477	9043
	2260	4586	9006
	2264	4670	8668
	2255	4477	9043
	2210	4622	8873
	2242	4550	8946
Mean	2222 (v=10.4)	4556 (v=3.4)	8950 (v=4.7)
9	2192	4405	8680
	2099	4417	9067
	2210	4562	9079
	2130	4308	9103
	2242	4610	9103
	2260	4610	9079
	2237	4550	9115
	2188	4429	9006
	2228	4525	8886
Mean	2198 (v=7.3)	4491 (v=6.7)	9013 (v=4.8)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
10	2130	4079	8596
	2148	4187	8898
	2174	4368	8910
	2242	4501	9175
	2264	4393	9018
	2309	4489	8946
	2179	4368	8801
	2125	4079	8656
	2201	4006	8596
Mean	2197 (v=8.4)	4274 (v=11.6)	8844 (v=6.5)
11	2139	4296	8741
	2179	4260	9091
	2219	4006	8934
	2192	4381	8704
	2042	4284	8656
	2152	4175	8704
	2152	4163	8173
	2174	4344	8197
	2165	4332	8946
Mean	2157 (v=8.2)	4249 (v=8.8)	8683 (v=10.6)
12	2210	4453	8523
	2192	4465	9079
	2260	4550	9103
	2246	4550	9139
	2197	4344	9139
	2305	4538	8982
	2296	4562	9055
	2255	4477	9067
	2278	4417	8970
Mean	2249 (v=5.0)	4484 (v=4.9)	9006 (v=6.8)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
13	2201	4320	8668
	2264	4574	8934
	2264	4453	8608
	2278	4550	8946
	2296	4707	8994
	2269	4248	8934
	2255	4634	8753
	2255	4525	9018
	2260	4852	9103
Mean	2260 (v=4.2)	4540 (v=13.3)	8884 (v=5.6)
14	2219	4574	8523
	2255	4562	8886
	2255	4393	8753
	2314	4538	8946
	2264	4477	8849
	2237	4489	9079
	2210	4465	9115
	2237	4405	8946
	2233	4320	8777
Mean	2247 (v=4.6)	4469 (v=5.7)	8875 (v=6.7)
15	2099	3861	8185
	2219	4538	8813
	2210	4538	9103
	2188	4296	8898
	2224	4574	9006
	2242	4381	9067
	2278	4525	8813
	2210	4682	8970
	2246	4658	9091
Mean	2213 (v=8.1)	4450 (v=18.4)	8883 (v=10.3)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
16	2121	4284	8584
	2192	4332	8777
	2165	4381	8729
	2179	4127	8994
	2206	4199	8813
	2233	4441	9151
	2251	4453	8910
	2170	4320	8837
	2042	4381	9079
Mean	2173 (v=9.6)	4324 (v=7.5)	8875 (v=6.4)
17	2233	4501	8922
	2255	4538	8910
	2251	4489	9248
	2282	4489	8898
	2233	4344	8958
	2273	4477	8801
	2224	4465	9043
	2237	4658	9030
	2264	4344	8777
Mean	2250 (v=2.6)	4478 (v=7.0)	8954 (v=5.3)
18	2282	4501	8535
	2219	4465	8898
	2269	4562	8934
	2233	4695	9067
	2287	4405	8873
	2278	4574	8910
	2260	4405	8910
	2287	4574	8886
	2246	4513	9079
Mean	2262 (v=3.0)	4522 (v=6.4)	8899 (v=6.1)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 3

(Analysed concentration of HFC 143 - continued)

Exposure no.	Group		
	2 (Low dose HFC 143)	3 (Inter dose HFC 143)	4 (High dose HFC 134)
19	2201	4536	8704
	2278	4586	9115
	2228	4550	8886
	2224	4489	8994
	2251	4550	8825
	2255	4731	8982
	2251	4441	9043
	2228	4550	9067
	2233	4332	8934
Mean	2239 (v=3.4)	4529 (v=8.8)	8950 (v=4.6)
20	2143	4441	8596
	2233	4405	8668
	2246	4513	8680
	2242	4417	8898
	2210	4538	9103
	2242	4550	8873
	2264	4453	8958
	2210	4501	8982
	2174	4381	8970
Mean	2218 (v=5.5)	4467 (v=3.8)	8859 (v=5.7)
21	2148	4344	8572
	2219	4441	9006
	2255	4513	8813
	2246	4332	8632
	2255	4670	9006
	2228	4308	8680
	2260	4598	8777
	2206	4562	8970
	2269	4417	8994
Mean	2232 (v=5.4)	4465 (v=8.1)	8828 (v=4.9)

v Variation = $\frac{\text{range}}{\text{mean}} \times 100\%$

APPENDIX 4

Individual clinical signs - between exposures

Group	Rat no.	Observation
1 (Air Control)	1♂	NAD
	2♂	NAD
	3♂	NAD
	4♂	NAD
	5♂	NAD
	6♂	NAD
	7♂	NAD
	8♂	NAD
	9♂	NAD
	10♂	NAD
2 (Low dose)	11♂	NAD
	12♂	NAD
	13♂	NAD
	14♂	NAD
	15♂	NAD
	16♂	NAD
	17♂	NAD
	18♂	NAD
	19♂	NAD
	20♂	Brown staining on head, Days 43 and 44
3 (Inter dose)	21♂	NAD
	22♂	Brown staining on head Day 15
	23♂	NAD
	24♂	Noisy respiration Day 2
	25♂	Brown staining on head Day 15
	26♂	NAD
	27♂	Noisy respiration Day 2, lethargic Day 3
	28♂	NAD
	29♂	Brown staining on head Day 15
	30♂	NAD

NAD Nothing abnormal detected

APPENDIX 4

(Individual clinical signs - continued)

Group	Rat no.	Observation
4 (High dose)	31♂	NAD
	32♂	Lethargic Days 2 and 5, pale extremities Days 2 and 5, brown staining on head Day 8
	33♂	Lethargic Days 2, 3, 4 and 5, pale extremities Days 2, 3 and 5, brown staining on head Day 8, cold to touch Days 2 and 3, ataxia Day 2, exaggerated breathing Day 3, poorly groomed Day 5
	34♂	Lethargic Days 2 to 5, pale extremities Days 2, 3 and 5, cold to touch Days 2, 3, 5 and 6 ataxia Day 2, exaggerated breathing Day 3, poorly groomed Day 5, occasional body tremors Day 5, piloerection Day 6, hunched posture Day 6. Found dead Day 7
	35♂	Lethargic Days 2, 3 and 5, pale extremities Days 3 and 6, red/brown staining around snout Day 3
	36♂	Lethargic Days 2, 3 and 5, pale extremities Days 2, 3 and 5, red/brown staining around snout Day 3, state of collapse Day 19, red/brown staining around snout Day 19, exaggerated breathing Day 19, occasional paralysis of hindlimbs Day 19, area of opacity in both eyes Day 19, red/brown staining on head Day 20
	37♂	Lethargic, pale extremities, cold to touch, ataxia, piloerection and exaggerated breathing Days 2 and 3. Found dead Day 4
	38♂	Lethargic Days 2, 3 and 5, ataxia Days 2 and 3, pale extremities Day 5, brown staining on head Days 8, 15, 20 and 21, convulsion (lasting up to 20 seconds) Day 19, muscular spasms Day 19, state of collapse Day 19, red/brown staining around snout Day 20.
	39♂	Lethargic Days 2 to 5, pale extremities Days 2 and 3, brown staining on head Days 8 and 15
	40♂	Poorly groomed Day 5

NAD Nothing abnormal detected

APPENDIX 4

(Individual clinical signs - continued)

Group	Rat no.	Observation
1 (Air Control)	41♀	NAD
	42♀	NAD
	43♀	NAD
	44♀	NAD
	45♀	NAD
	46♀	NAD
	47♀	Brown staining on head, Days 22 to 28
	48♀	NAD
	49♀	NAD
	50♀	NAD
2 (Low dose)	51♀	NAD
	52♀	NAD
	53♀	NAD
	54♀	NAD
	55♀	NAD
	56♀	Brown staining on head, Days 22 to 28
	57♀	Brown staining on head, Days 22 to 32
	58♀	NAD
	59♀	Brown staining on head, Days 22 to 24
	60♀	NAD
3 (Inter dose)	61♀	Brown staining on head, Days 22 to 28
	62♀	Lethargic Day 2, pale extremities Days 2 and 3, hair loss from head Days 22 to 30, ataxia Day 26
	63♀	Lethargic Days 2 and 3, pale extremities Days 2 and 3, brown staining on head Days 22 to 24, ataxia Day 26. Two convulsions of 20 seconds duration Day 26
	64♀	Hair loss from head Day 29 and 30
	65♀	NAD
	66♀	NAD
	67♀	NAD
	68♀	Lethargic Days 2, 3 and 5, pale extremities Days 2, 3 and 5, cold to touch Day 2
	69♀	Lethargic Days 2 and 3, pale extremities Days 2 and 3. Cold to touch Days 2 and 3
	70♀	Lethargic Day 2, pale extremities Day 2, left forelimb digit damaged Day 36

NAD Nothing abnormal detected

APPENDIX 4

(Individual clinical signs - continued)

Group	Rat no.	Observation
4 (High dose)	71♀	Pale extremities Day 2, lethargic Day 5
	72♀	Lethargic Days 2 and 5, pale extremities Days 2, 3, 5 and 15, ataxia Day 2, cold to touch Day 15, exaggerated breathing Day 15, brown staining on head Days 15 and 20
	73♀	Ataxia Day 2, noisy respiration Day 2, lethargic Days 2, 3 and 5, pale extremities Days 2, 3 and 5, cold to touch Days 2 and 3, exaggerated breathing Days 2 and 3, red/brown staining around snout Day 3, poorly groomed Day 5.
	74♀	Lethargic Days 2 to 5, pale extremities Days 2 and 3, cold to touch Days 2 and 3, exaggerated breathing Days 2 and 3
	75♀	Lethargic Day 2, pale extremities Day 2, cold to touch Day 2, ataxia Day 2, exaggerated breathing Day 2. Found dead Day 3
	76♀	Lethargic Days 2 to 4, pale extremities Days 2 to 4, cold to touch Days 2 to 4, ataxia Days 2 and 3, piloerection Days 2 and 3, occasional paralysis of hindlimbs Day 4. Found dead Day 5
	77♀	Lethargic Days 2, 3 and 5, pale extremities Days 2 and 3, cold to touch Days 2 and 3, ataxia Days 2 and 3, exaggerated breathing Days 2 and 3, red/brown staining around snout Day 3. Found dead Day 19
	78♀	Lethargic Days 2 to 7, pale extremities Days 2 to 7, cold to touch Days 2 to 7, piloerection Days 2 to 7, occasional body tremors Days 2 and 5, yellow staining urogenital region Days 4 and 5, hunched posture Days 6 and 7. Found dead Day 7
	79♀	Lethargic Days 2 and 3, pale extremities Days 2 and 3, cold to touch Days 2 and 3, ataxia Days 2 and 3, exaggerated breathing Day 3, red/brown staining around snout Day 3. Found dead Day 4.
	80♀	Lethargic Days 2 and 6, pale extremities Days 2 and 6, cold to touch Days 2 and 6, piloerection Days 2 to 6, exaggerated breathing Days 3 to 5, hunched posture Days 2 to 6, occasional body tremors Day 5. Found dead Day 7

NAD Nothing abnormal detected

APPENDIX 5

Bodyweights - individual values (g)

Group 1♂: Air Control

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
1	169	200	230	*256	266	271	294	320	323	346	352				
2	176	214	245	283	305	328	339	367	385	402	417				
3	171	209	240	274	291	317	328	357	367	388	402				
4	168	201	235	262	279	308	320	345	356	373	394				
5	180	210	240	270	291	312	320	342	354	365	379				
6	176	211	239	265	278	297	311	323	332	346	359	354	357	364	373
7	172	200	227	261	280	304	314	334	354	369	385	384	403	419	434
8	166	200	228	255	275	289	300	319	332	343	362	365	375	390	400
9	171	205	231	268	288	310	325	349	361	377	387	385	404	416	425
10	175	211	237	261	284	310	323	350	363	381	406	414	433	448	458

Group 2♂: 2250 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
11	176	203	227	250	261	282	291	311	327	348	358				
12	172	204	235	262	279	305	320	335	347	360	375				
13	169	200	228	254	276	303	314	341	347	364	379				
14	173	213	248	278	301	326	338	361	372	400	415				
15	175	207	239	271	291	312	336	355	368	391	401				
16	171	199	226	235	254	276	294	321	334	357	369	377	391	407	422
17	177	216	244	275	300	325	341	360	372	387	402	401	417	426	434
18	177	213	246	276	302	332	343	366	378	391	404	411	425	440	452
19	167	199	225	244	258	277	290	310	319	344	353	351	365	373	387
20	169	215	247	273	290	312	321	340	356	375	395	395	408	424	435

APPENDIX 5

(Bodyweights - continued)

Group 3♂: 4500 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
21	166	196	223	237	266	297	308	336	354	375	390				
22	174	194	225	236	246	270	276	290	302	318	332				
23	182	219	251	271	284	299	310	326	334	349	360				
24	171	200	230	252	280	308	326	353	370	387	395				
25	170	207	232	257	278	307	321	345	362	388	399				
26	174	213	248	271	292	317	331	366	372	400	402	414	435	451	469
27	166	189	211	208	231	258	268	295	310	331	347	352	368	379	384
28	163	201	229	249	265	289	294	319	324	351	364	370	383	394	407
29	171	203	233	255	268	291	301	325	341	357	369	368	378	394	397
30	175	220	255	260	278	309	324	344	365	392	405	404	430	442	459

Group 4♂: 9000 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
31	172	203	228	247	260	277	287	307	316	336	354				
32	179	217	248	266	280	307	319	345	359	380	396				
33	167	201	230	199	230	263	265	288	299	316	319				
34	172	211	237	191											
35	175	204	235	232	246	282	289	315	324	344	352				
36	170	201	225	202	226	254	249	279	278	307	317	319	339	341	357
37	170	204	229												
38	173	205	231	225	234	267	267	292	281	298	307	310	326	337	346
39	177	212	238	229	249	286	297	323	337	360	374	379	388	402	416
40	177	204	226	245	254	271	275	289	300	320	332	325	341	355	362

APPENDIX 5

(Bodyweights - continued)

Group 1♀: Air Control

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
41	142	163	185	205	221	212	233	245	249	263	260				
42	142	162	177	193	213	216	225	236	241	244	243				
43	140	160	177	200	190	214	227	246	247	257	266				
44	151	177	191	209	217	231	231	243	259	265	280				
45	139	157	174	191	193	206	213	226	229	241	244				
46	153	177	201	227	233	253	255	276	278	297	298	283	304	316	324
47	136	162	180	198	206	223	216	233	247	253	266	267	270	284	278
48	141	167	185	202	201	226	218	249	251	273	270	275	281	292	304
49	130	147	162	167	182	192	200	216	220	227	227	225	238	246	248
50	142	161	177	195	209	221	227	241	249	265	274	269	284	291	297

Group 2♀: 2250 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
51	129	145	151	187	201	214	229	241	242	248	262				
52	137	159	175	189	192	205	212	231	240	245	255				
53	127	154	175	190	204	217	222	230	248	252	262				
54	132	151	162	177	182	188	200	218	220	232	242				
55	144	165	170	186	203	224	221	223	246	254	240				
56	142	157	169	185	197	211	221	237	245	255	252	257	267	275	280
57	137	160	172	188	189	198	211	225	234	243	248	246	240	250	258
58	153	180	197	221	237	249	259	278	284	294	295	290	303	313	319
59	131	150	166	174	182	194	208	220	224	232	238	240	243	248	255
60	145	172	189	202	212	229	237	250	246	253	278	270	287	288	288

APPENDIX 5

(Bodyweights - continued)

Group: 3♀ 4500 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
61	151	172	195	206	206	217	235	253	253	254	272				
62	143	151	169	168	182	194	189	211	221	237	237				
63	132	153	172	174	186	210	220	254	249	260	258				
64	142	169	189	204	218	226	240	255	247	265	278				
65	151	169	194	207	215	228	229	247	245	259	256				
66	143	166	188	200	204	221	227	239	239	239	259	259	261	272	266
67	142	165	185	193	202	215	216	231	232	248	249	250	256	268	276
68	147	162	172	174	185	193	204	216	225	238	241	239	245	254	255
69	136	151	171	170	188	204	217	238	242	253	258	258	270	279	280
70	134	154	171	181	190	200	202	219	226	241	243	246	247	263	265

Group: 4♀ 9000 ppm HFC 143

Animal number	Week														
	-1	-0.3	0	0.4	1	1.4	2	2.4	3	3.4	4	4.4	5	5.4	6
71	127	150	162	174	185	191	194	210							
72	145	164	182	183	197	212	214	228							
73	137	159	175	167	189	205	214	225							
74	137	159	170	160	183	201	211	227							
75	127	150	171												
76	137	158	170												
77	128	144	160	153	169	183	196	215							
78	139	163	179	159											
79	154	173	186												
80	137	161	174	149											

APPENDIX 6

Food consumption - individual values (g/rat/week)

Group: 1♂ Air Control

Week	Animal									
	1	2	3	4	5	6	7	8	9	10
-0.4	107	113	115	108	108	113	95	103	111	113
-0.1	84	92	95	89	89	92	82	84	87	91
0.4	108	128	124	120	124	129	119	119	127	127
1	79	100	94	94	95	94	94	98	99	104
1.4	108	122	118	122	118	122	125	123	124	133
2	78	86	84	91	83	92	80	90	90	99
2.4	109	122	119	121	115	115	109	117	126	140
3	78	93	91	89	82	82	87	91	95	101
3.4	116	125	124	124	119	121	122	123	125	133
4	78	90	90	94	83	85	87	91	81	104
Withdrawal										
4.4						108	113	120	116	140
5						90	96	100	101	115
5.4						109	132	135	137	153
6						80	92	98	95	102

APPENDIX 6

(Food consumption - continued)

Group: 2♂ 2250 ppm HFC 143

Week	Animal									
	11	12	13	14	15	16	17	18	19	20
-0.4	99	114	114	117	111	107	118	110	98	120
-0.1	80	96	90	99	98	89	93	87	82	110
0.4	102	121	109	125	123	86	129	117	96	140
1	74	89	89	102	94	76	101	99	72	98
1.4	107	125	125	139	115	110	129	127	101	132
2	70	88	82	98	91	83	90	89	69	88
2.4	110	120	129	142	123	128	128	120	106	129
3	83	85	89	97	91	86	95	81	73	94
3.4	119	116	121	145	130	128	125	122	116	148
4	83	82	87	97	92	91	90	93	76	100
Withdrawal										
4.4						120	121	119	103	140
5						103	104	107	88	109
5.4						117	133	135	110	141
6						87	91	98	80	104

APPENDIX 6

(Food consumption - continued)

Group: 3♂ 4500 ppm HFC 143

Week	Animal									
	21	22	23	24	25	26	27	28	29	30
-0.4	90	99	111	107	107	118	99	111	113	127
-0.1	78	87	97	90	86	93	79	88	93	107
0.4	83	80	114	106	100	112	61	105	110	87
1	88	64	89	95	84	92	83	85	88	88
1.4	124	97	108	131	124	129	119	116	117	123
2	81	60	69	92	88	91	79	71	87	83
2.4	119	99	108	133	122	136	117	117	125	121
3	97	71	75	86	93	93	85	78	90	91
3.4	124	100	112	126	136	145	122	132	127	130
4	87	69	70	82	87	84	89	88	81	85
Withdrawal										
4.4						130	128	129	118	123
5						113	96	106	107	107
5.4						150	126	133	134	134
6						100	78	96	88	95

APPENDIX 6

(Food consumption - continued)

Group: 4♂ 9000 ppm HFC 143

Week	Animal									
	31	32	33	34	35	36	37	38	39	40
-0.4	101	128	107	114	108	108	109	108	107	100
-0.1	82	102	90	88	85	87	84	89	88	77
0.4	89	103	34	11	65	38	(0)	57	62	106
1	76	83	70	13	62	72		62	76	77
1.4	111	133	127		127	114		125	125	108
2	75	85	56		75	55		64	78	70
2.4	105	135	112		130	114		120	122	109
3	74	98	80		85	55		49	86	75
3.4	114	146	109		123	128		115	126	119
4	83	104	68		86	81		78	84	72
Withdrawal										
4.4						109		102	114	106
5						105		104	92	97
5.4						115		133	124	119
6						84		85	86	82

( ) Value excluded from means and statistical analysis

APPENDIX 6

(Food consumption - continued)

Group: 1♀ Air Control

Week	Animal									
	41	42	43	44	45	46	47	48	49	50
-0.4	81	80	80	94	77	92	87	78	75	83
-0.1	67	64	64	70	66	79	67	64	60	69
0.4	91	85	86	90	85	107	95	88	80	92
1	70	71	62	75	64	81	75	68	63	88
1.4	72	75	88	91	83	107	97	102	77	94
2	57	52	72	70	54	62	65	64	57	65
2.4	96	85	95	95	80	101	99	102	96	97
3	56	58	59	77	51	73	68	70	64	70
3.4	100	81	99	96	90	114	97	107	93	108
4	52	55	65	79	61	71	78	68	68	76
Withdrawal										
4.4						97	96	101	87	86
5						92	80	84	79	88
5.4						117	118	111	93	106
6						89	71	86	67	78

APPENDIX 6

(Food consumption - continued)

Group: 2♀ 2250 ppm HFC 143

Week	Animal									
	51	52	53	54	55	56	57	58	59	60
-0.4	78	92	77	76	88	84	86	91	74	88
-0.1	70	51	69	67	59	64	66	73	60	72
0.4	95	81	81	72	89	82	84	96	71	84
1	76	61	63	56	71	62	63	80	55	63
1.4	102	84	79	69	98	85	88	98	77	87
2	68	67	54	52	74	62	64	72	60	66
2.4	105	89	82	84	92	93	91	110	83	94
3	67	68	69	55	81	70	69	78	59	65
3.4	105	93	88	93	99	96	100	104	85	95
4	81	65	60	63	66	63	64	72	57	74
Withdrawal										
4.4						87	86	92	79	95
5						79	70	87	65	81
5.4						99	96	108	78	95
6						70	68	78	61	68

APPENDIX 6

(Food consumption - continued)

Group: 3♀ 4500 ppm HFC 143

Week	Animal									
	61	62	63	64	65	66	67	68	69	70
-0.4	90	68	80	81	87	87	85	83	77	80
-0.1	74	57	68	69	75	72	67	62	68	67
0.4	84	40	45	84	85	79	66	55	53	66
1	68	53	64	70	72	60	57	56	63	57
1.4	84	74	94	83	96	88	84	74	100	82
2	70	32	50	66	63	62	52	59	63	55
2.4	100	81	113	94	93	92	84	89	105	88
3	68	56	61	60	67	70	58	63	65	61
3.4	101	83	94	103	98	97	87	93	102	93
4	69	44	46	65	66	71	55	59	63	57
Withdrawal										
4.4						98	84	84	108	91
5						47	75	74	87	71
5.4						95	96	86	108	101
6						64	65	66	74	71

APPENDIX 6

(Food consumption - continued)

Group: 4♀ 9000 ppm HFC 143

Week	Animal									
	71	72	73	74	75	76	77	78	79	80
-0.4	85	80	85	81	80	78	75	82	78	73
-0.1	64	67	67	61	67	61	63	67	58	63
0.4	71	50	39	29		25	37	15		6
1	59	63	55	55			55	10		5
1.4	78	88	95	97			89			
2	54	56	56	51			55			
2.4	89	89	96	103			92			
3	24	24	23	6						
3.4										
4										
Withdrawal										
4.4										
5										
5.4										
6										

APPENDIX 7

Water consumption - individual values (g/rat/day)

Group 1♂: Air Control

Week	Animal									
	1	2	3	4	5	6	7	8	9	10
-1	40	29	28	32	22	32	26	35	29	42
-0.6	44	31	31	39	26	36	29	38	28	37
-0.5	43	32	32	35	25	40	27	35	27	35
-0.4	40	28	32	31	27	38	29	35	25	37
-0.3	37	31	32	31	24	41	33	33	24	35
-0.2	43	30	32	28	27	37	28	34	27	40
-0.1	43	35	33	30	29	39	32	33	26	42
0.1	42	32	33	32	30	40	32	35	29	38
0.2	37	32	30	33	28	42	32	32	30	32
0.3	40	31	29	30	24	37	31	32	28	37
0.4	42	30	32	27	27	35	32	34	30	45
0.5	38	30	30	30	27	36	32	35	29	43
0.6	38	30	28	28	27	35	29	31	31	40
1	40	30	30	24	27	37	32	35	32	38
1.1	39	28	28	27	28	42	30	32	28	31
1.2	46	30	33	29	28	42	33	34	32	34
1.3	31	26	22	25	20	33	20	29	21	35
1.4	41	30	30	29	24	34	29	33	27	39
1.5	35	24	24	24	22	32	21	30	25	29
1.6	40	29	31	28	27	38	29	37	28	35
2	33	24	25	26	21	37	22	25	27	32
2.1	38	32	32	27	24	35	20	30	25	35
2.2	41	31	32	28	27	34	28	32	23	36
2.3	38	29	33	27	22	36	25	32	30	31
2.4	44	26	31	25	26	39	27	29	33	31

APPENDIX 7

(Water consumption - continued)

Group 1♂: Air Control

Week	Animal									
	1	2	3	4	5	6	7	8	9	10
2.5	27	24	24	25	22	25	25	31	23	30
2.6	45	33	34	35	27	44	29	37	33	36
3	39	30	27	26	25	35	27	34	28	32
3.1	37	27	28	30	23	33	23	33	31	36
3.2	33	32	30	29	27	30	28	31	35	36
3.3	47	40	34	29	28	39	30	33	37	34
3.4	52	34	33	28	26	33	29	31	33	37
3.5	31	25	23	29	20	25	24	33	20	38
3.6	31	27	26	30	21	28	24	33	24	35
4	47	33	35	28	31	45	32	33	34	39
Withdrawal										
4.1						29	24	27	22	33
4.2						42	45	43	43	52
4.3						38	26	29	33	35
4.4						34	33	40	34	40
4.5						34	34	26	33	36
4.6						33	32	34	36	38
5						34	31	33	38	36
5.1						36	31	42	43	44
5.2						32	32	35	37	43
5.3						38	27	35	39	38
5.4						40	30	34	33	34
5.5						23	22	34	18	29
5.6						30	46	30	41	43
6						40	29	33	33	36

APPENDIX 7

(Water consumption - continued)

Group 2♂: 2250 ppm HFC 143

Week	Animal									
	11	12	13	14	15	16	17	18	19	20
-1	23	41	27	31	24	28	39	33	26	31
-0.6	29	37	30	30	26	29	39	31	27	31
-0.5	24	40	28	33	26	29	35	33	27	32
-0.4	27	37	27	31	25	28	35	30	25	33
-0.3	27	34	25	32	26	26	35	28	24	32
-0.2	28	35	28	35	28	28	36	30	23	31
-0.1	30	35	27	32	28	27	36	32	26	35
0.1	29	33	28	35	28	20	36	31	24	34
0.2	26	31	29	34	28	25	37	28	25	31
0.3	29	35	27	30	27	27	33	30	21	33
0.4	33	35	27	33	29	27	38	32	30	31
0.5	29	33	28	35	22	27	34	31	23	31
0.6	29	29	29	34	27	27	32	31	22	28
1	24	31	32	33	27	26	34	33	26	29
1.1	24	30	31	33	25	31	30	33	27	29
1.2	28	33	32	34	26	30	32	32	23	31
1.3	22	26	21	24	21	19	23	20	19	26
1.4	27	28	33	32	25	27	34	37	26	30
1.5	20	22	28	25	28	20	25	22	21	25
1.6	25	31	33	31	29	31	33	34	23	29
2	25	31	28	31	23	26	30	27	23	25
2.1	25	27	29	28	27	31	32	33	21	28
2.2	29	30	33	33	29	31	31	33	27	34
2.3	24	30	29	30	22	31	33	28	24	26
2.4	27	34	30	29	26	32	30	32	24	31

APPENDIX 7

(Water consumption - continued)

Group 2♂: 2250 ppm HFC 143

Week	Animal									
	11	12	13	14	15	16	17	18	19	20
2.5	21	22	20	24	19	17	23	8	13	18
2.6	32	33	37	35	32	34	35	40	31	39
3	27	25	28	29	26	29	30	29	26	31
3.1	31	31	33	33	27	32	32	27	25	30
3.2	31	28	32	31	28	28	33	24	19	30
3.3	36	31	38	36	27	33	32	30	26	37
3.4	45	31	34	32	25	27	31	35	29	36
3.5	29	26	28	23	22	20	23	19	17	25
3.6	31	26	27	27	28	24	28	30	21	32
4	30	29	33	38	29	33	35	35	30	38
Withdrawal										
4.1						(186)	24	19	19	28
4.2						57	44	49	35	43
4.3						35	31	36	27	34
4.4						34	34	32	25	40
4.5						32	23	33	28	32
4.6						34	33	35	29	38
5						34	38	32	26	34
5.1						33	33	39	25	36
5.2						31	32	37	28	35
5.3						26	31	30	25	35
5.4						27	31	29	23	32
5.5						20	24	20	19	26
5.6						32	32	40	33	36
6						32	30	29	25	33

( ) Leak from water bottle. Value excluded from means and statistical analysis

APPENDIX 7

(Water consumption - continued)

Group 3♂: 4500 ppm HFC 143

Week	Animal									
	21	22	23	24	25	26	27	28	29	30
-1	25	28	29	33	22	30	28	47	31	27
-0.6	29	32	30	33	28	33	32	44	30	30
-0.5	26	30	32	35	26	36	28	41	32	29
-0.4	23	27	31	33	26	36	27	40	28	29
-0.3	27	34	35	34	24	32	28	37	31	31
-0.2	27	33	35	32	29	41	26	41	31	31
-0.1	26	30	33	34	27	40	27	35	29	34
0.1	22	22	31	53	24	33	29	48	37	19
0.2	14	22	35	53	23	46	6	65	32	11
0.3	35	39	33	62	27	45	31	57	42	31
0.4	31	33	35	52	28	40	40	51	49	30
0.5	30	31	34	58	29	37	31	53	41	28
0.6	29	24	28	55	27	42	23	61	23	24
1	29	26	30	37	29	39	23	56	47	24
1.1	28	24	30	48	31	46	27	59	39	28
1.2	32	28	34	37	31	38	23	61	40	26
1.3	25	22	23	28	27	30	24	51	31	22
1.4	27	28	23	34	27	34	27	48	36	25
1.5	22	18	26	27	28	30	23	44	31	19
1.6	27	35	29	38	29	32	25	56	35	24
2	26	27	27	32	26	26	23	51	29	23
2.1	27	26	30	33	28	32	23	40	31	23
2.2	30	28	28	30	31	35	26	46	34	25
2.3	27	23	28	32	29	34	27	42	30	21
2.4	32	28	30	32	28	40	27	43	38	23

APPENDIX 7

(Water consumption - continued)

Group 3♂: 4500 ppm HFC 143

Week	Animal									
	21	22	23	24	25	26	27	28	29	30
2.5	18	22	16	11	23	18	20	34	28	18
2.6	34	25	36	37	32	37	29	48	38	29
3	29	24	28	32	28	35	24	42	35	21
3.1	35	30	34	26	35	41	30	57	44	25
3.2	31	21	29	33	32	31	22	50	40	23
3.3	31	25	33	31	39	45	28	59	39	30
3.4	37	25	34	33	39	45	30	50	96	31
3.5	26	18	19	17	24	20	24	38	33	18
3.6	28	21	28	21	30	28	25	37	35	29
4	34	27	31	33	33	35	31	41	36	30
Withdrawal										
4.1						30	21	42	31	22
4.2						48	42	55	47	43
4.3						46	26	52	37	29
4.4						45	31	53	41	23
4.5						51	27	53	37	35
4.6						43	28	52	35	35
5						46	29	47	35	30
5.1						53	31	53	43	23
5.2						51	30	44	33	35
5.3						45	27	47	41	33
5.4						41	28	43	38	31
5.5						27	22	36	28	26
5.6						51	31	44	35	36
6						47	28	41	33	31

APPENDIX 7

(Water consumption - continued)

Group 4♂: 9000 ppm HFC 143

Week	Animal									
	31	32	33	34	35	36	37	38	39	40
-1	22	37	26	27	28	32	36	27	27	30
-0.6	26	39	29	30	30	31	36	32	35	33
-0.5	23	37	33	35	33	31	34	27	36	34
-0.4	23	35	30	33	31	26	30	31	33	33
-0.3	25	41	30	33	32	30	35	28	32	31
-0.2	21	39	33	40	35	27	29	29	39	26
-0.1	26	45	32	35	30	28	30	29	35	34
0.1	20	36	12	14	32	13	8	17	45	31
0.2	15	23	17	6	9	14	3	5	7	25
0.3	29	53	14	16	51	18	2	37	70	31
0.4	31	58	50	28	42	31		48	83	36
0.5	26	36	50	31	37	38		43	68	29
0.6	27	40	34	6	36	31		53	58	30
1	27	39	34		41	35		47	52	32
1.1	25	37	33		34	31		47	60	33
1.2	27	38	33		32	31		45	57	29
1.3	21	33	30		27	25		31	40	27
1.4	24	37	30		37	35		37	48	36
1.5	17	27	4		23	14		17	37	24
1.6	26	37	31		44	34		46	54	33
2	21	35	28		37	42		38	50	31
2.1	23	39	28		35	44		37	39	34
2.2	24	40	25		31	37		37	42	22
2.3	21	39	28		31	38		23	38	33
2.4	24	38	29		35	33		35	39	27

APPENDIX 7

(Water consumption - continued)

Group 4♂: 9000 ppm HFC 143

Week	Animal									
	31	32	33	34	35	36	37	38	39	40
2.5	19	26	15		20	16		21	30	19
2.6	22	47	30		37	31		33	45	37
3	22	42	29		29	30		42	38	34
3.1	26	45	27		33	41		40	47	34
3.2	20	43	26		32	31		32	41	31
3.3	26	41	32		38	39		33	40	38
3.4	26	43	31		36	37		45	41	36
3.5	22	36	16		24	30		39	31	22
3.6	22	32	25		26	34		38	34	26
4	28	47	32		35	34		43	42	37
Withdrawal										
4.1						25		24	38	26
4.2						64		45	53	43
4.3						41		45	42	36
4.4						33		47	43	38
4.5						36		38	39	38
4.6						35		40	54	39
5						33		39	39	42
5.1						28		41	47	41
5.2						59		40	48	48
5.3						26		33	38	47
5.4						29		33	38	49
5.5						32		33	30	26
5.6						32		39	48	44
6						29		31	41	42

APPENDIX 7

(Water consumption - continued)

Group 1♀: Air Control

Week	Animal									
	41	42	43	44	45	46	47	48	49	50
-1	19	23	23	26	34	33	22	26	29	24
-0.6	25	36	29	27	34	36	26	28	24	26
-0.5	22	26	26	26	31	40	26	24	22	21
-0.4	24	28	26	28	30	33	25	27	28	24
-0.3	23	29	26	24	29	34	21	25	28	23
-0.2	24	27	24	27	29	33	24	18	26	24
-0.1	27	26	30	29	28	36	29	31	24	22
0.1	29	30	30	28	31	34	25	29	29	25
0.2	25	26	28	24	26	29	23	20	24	22
0.3	26	24	20	26	22	34	24	12	22	22
0.4	25	19	34	27	27	37	25	31	19	22
0.5	27	27	24	27	24	33	24	27	26	26
0.6	24	24	28	24	23	30	21	23	24	24
1	26	24	16	27	22	29	24	12	26	24
1.1	24	19	31	28	26	35	29	36	22	25
1.2	28	26	23	26	24	32	20	26	27	25
1.3	5	10	15	18	13	25	18	13	16	16
1.4	19	19	23	28	23	31	26	28	26	25
1.5	8	2	22	22	19	19	15	18	15	16
1.6	34	30	28	27	21	35	25	25	33	25
2	24	21	23	18	20	30	13	6	25	23
2.1	26	19	16	29	21	36	24	25	26	22
2.2	25	38	32	25	26	34	23	27	25	26
2.3	26	33	25	24	25	31	22	13	29	25
2.4	26	28	28	19	26	43	19	23	30	29

APPENDIX 7

(Water consumption - continued)

Group 1♀: Air Control

Week	Animal									
	41	42	43	44	45	46	47	48	49	50
2.5	4	2	10	25	8	18	16	18	13	16
2.6	39	31	36	23	29	45	29	23	25	26
3	26	28	25	25	26	36	19	14	30	24
3.1	25	23	26	23	23	29	15	31	26	29
3.2	27	47	21	22	20	33	26	12	25	26
3.3	27	27	29	24	29	34	21	19	22	27
3.4	29	31	27	25	25	39	20	29	30	30
3.5	7	10	18	21	15	21	17	8	22	21
3.6	30	29	21	26	21	28	31	26	27	32
4	32	23	34	27	29	37	24	22	24	32
Withdrawal										
4.1						21	19	17	16	22
4.2						45	27	30	39	37
4.3						37	27	27	31	31
4.4						37	28	26	26	34
4.5						44	29	25	34	32
4.6						38	25	27	32	33
5						40	27	22	28	36
5.1						39	28	33	26	39
5.2						37	28	27	34	34
5.3						44	22	25	32	29
5.4						38	26	20	30	36
5.5						31	24	22	20	30
5.6						38	23	32	33	37
6						35	14	25	33	37

APPENDIX 7

(Water consumption - continued)

Group 2♀: 2250 ppm HFC 143

Week	Animal									
	51	52	53	54	55	56	57	58	59	60
-1	29	27	28	21	24	26	24	25	20	29
-0.6	31	28	31	20	15	26	25	24	22	31
-0.5	29	29	32	23	40	23	26	25	20	30
-0.4	27	28	29	22	31	23	26	25	21	29
-0.3	27	26	27	22	28	23	24	27	20	29
-0.2	29	25	31	20	22	24	20	29	22	31
-0.1	23	28	26	18	19	23	25	28	21	30
0.1	28	28	26	21	36	30	29	30	16	20
0.2	26	22	25	19	22	23	21	30	18	26
0.3	21	20	25	21	23	24	17	25	19	26
0.4	23	30	27	24	18	23	28	28	22	30
0.5	25	24	23	20	23	27	25	29	14	18
0.6	24	23	23	19	30	23	21	28	24	27
1	20	17	24	18	27	31	16	27	19	27
1.1	22	25	22	20	27	23	25	29	20	26
1.2	25	25	19	19	24	28	23	30	18	19
1.3	19	16	24	13	15	21	20	21	19	24
1.4	21	23	27	18	32	24	15	27	20	28
1.5	14	20	18	12	20	18	17	17	14	18
1.6	25	28	20	25	31	27	26	33	19	22
2	23	18	25	16	17	23	17	23	23	29
2.1	24	20	22	19	25	27	14	28	22	26
2.2	24	28	28	21	30	24	29	27	30	26
2.3	23	26	20	21	18	28	23	33	18	17
2.4	30	31	29	24	17	33	24	32	38	31

APPENDIX 7

(Water consumption - continued)

Group 2♀: 2250 ppm HFC 143

Week	Animal									
	51	52	53	54	55	56	57	58	59	60
2.5	10	13	21	11	26	23	15	22	16	15
2.6	27	32	29	20	31	20	26	30	57	32
3	25	24	28	21	22	32	26	26	37	19
3.1	28	23	26	25	31	26	23	26	33	30
3.2	23	20	17	21	18	23	17	23	71	23
3.3	22	35	31	23	37	25	27	29	53	29
3.4	32	31	28	20	37	29	27	33	50	21
3.5	23	14	15	13	26	23	19	21	19	26
3.6	23	22	22	17	21	26	16	27	24	26
4	31	32	28	25	16	22	26	26	(108)	32
Withdrawal										
4.1						15	14	24	22	15
4.2						34	34	36	24	38
4.3						32	17	29	25	41
4.4						29	27	32	35	43
4.5						33	25	35	29	30
4.6						27	22	32	22	38
5						29	16	33	28	39
5.1						27	31	35	28	34
5.2						30	25	40	96	31
5.3						30	19	30	49	30
5.4						28	19	35	47	36
5.5						31	18	34	29	31
5.6						31	23	40	63	30
6						26	23	41	72	32

( ) Leak from water bottle. Value excluded from means and statistical analysis

APPENDIX 7

(Water consumption - continued)

Group 3♀: 4500 ppm HFC 143

Week	Animal									
	61	62	63	64	65	66	67	68	69	70
-1	27	20	24	26	28	26	23	33	24	29
-0.6	23	26	27	30	32	30	26	35	29	37
-0.5	29	21	29	27	31	27	25	36	26	31
-0.4	26	18	25	26	28	25	24	34	23	31
-0.3	28	24	30	27	31	26	25	28	29	36
-0.2	25	23	27	31	33	27	28	26	29	33
-0.1	29	22	31	31	30	27	25	26	28	30
0.1	24	10	12	26	26	20	21	38	12	11
0.2	21	19	12	29	39	19	14	27	11	18
0.3	45	47	59	32	52	31	29	48	48	37
0.4	44	51	65	31	45	33	30	39	53	38
0.5	47	54	60	25	27	17	26	54	34	20
0.6	32	34	45	29	51	39	26	41	32	13
1	20	27	38	30	38	28	21	32	28	22
1.1	28	27	36	28	41	28	23	29	29	25
1.2	24	30	39	23	25	24	20	38	27	15
1.3	14	14	23	14	27	26	17	31	23	15
1.4	19	24	41	23	32	31	23	26	26	26
1.5	19	6	15	18	18	16	15	17	12	11
1.6	34	28	48	27	27	23	22	37	43	19
2	25	22	50	30	34	26	21	26	36	14
2.1	24	28	48	29	30	25	22	25	32	23
2.2	30	25	53	31	38	32	23	27	26	17
2.3	28	24	49	22	31	18	20	30	30	22
2.4	31	21	46	38	61	32	24	30	34	19

APPENDIX 7

(Water consumption - continued)

Group 3♀: 4500 ppm HFC 143

Week	Animal									
	61	62	63	64	65	66	67	68	69	70
2.5	19	18	22	14	35	30	17	20	15	12
2.6	36	22	56	35	135	28	23	35	28	23
3	33	26	51	20	64	28	22	33	29	17
3.1	33	23	48	21	32	29	20	33	34	23
3.2	27	20	38	33	55	30	18	25	27	19
3.3	31	30	53	39	49	26	27	27	26	23
3.4	26	31	47	29	42	21	27	35	32	25
3.5	24	13	12	19	26	29	33	26	16	9
3.6	28	22	33	23	55	28	21	37	32	18
4	36	29	50	35	87	28	24	41	32	25
Withdrawal										
4.1						17	18	21	19	14
4.2						40	33	36	49	28
4.3						36	27	26	40	26
4.4						44	30	28	38	30
4.5						29	30	37	31	28
4.6						20	32	30	34	28
5						nr	29	25	32	21
5.1						39	27	25	33	32
5.2						32	28	34	27	32
5.3						31	28	33	30	30
5.4						32	25	28	33	27
5.5						28	24	21	23	23
5.6						28	27	33	29	32
6						28	29	28	28	34

APPENDIX 7

(Water consumption - continued)

Group 4♀: 9000 ppm HFC 143

Week	Animal									
	71	72	73	74	75	76	77	78	79	80
-1	24	25	35	20	29	23	38	26	29	30
-0.6	27	25	33	23	30	22	27	33	27	35
-0.5	27	23	33	21	28	23	24	31	30	30
-0.4	28	21	33	23	26	23	23	29	28	29
-0.3	26	25	35	21	24	22	23	27	27	30
-0.2	27	27	34	21	22	16	26	30	21	33
-0.1	20	23	31	21	26	25	24	27	29	28
0.1	23	7	11	8	6	8	6	9	12	17
0.2	27	10	5	8	9	7	5	9	15	12
0.3	36	46	41	27		24	28	9	3	4
0.4	30	35	52	39		4	45	18		14
0.5	24	24	35	26			41	18		16
0.6	28	28	42	25			43	9		4
1	23	29	40	23			32			
1.1	20	27	41	25			35			
1.2	16	26	42	31			36			
1.3	22	22	48	23			27			
1.4	25	28	46	25			25			
1.5	17	18	44	13			32			
1.6	15	31	45	30			56			
2	25	30	53	29			50			
2.1	22	26	49	26			46			
2.2	27	39	39	31			58			
2.3	16	30	46	32			38			
2.4	28	25	37	33			39			
2.5	22	31	42	10						
2.6	8	8	10	11						
3	All	dead								

APPENDIX 8

Haematology - individual values

Week 3 (5 January 1994)

Group	Animal no.	PCV	Hb	RBC $\times 10^6$/	MCHC	MCV	MCH	Retic	WBC + Diff $\times 10^3/mm^3$						Film comments					Plts $\times 10^3$/	TT
		%	g/dl	mm^3	%	fl	pg	%	Total	N	L	E	B	M	P	H	A	R	S	mm^3	s
49	71	ctd	ctd	ctd	nr	nr	nr	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd	ctd
9000 ppm	72	48	14.9	6.4	31.0	75	23.3	1.8	4.7	0.38	4.18	0.14	0.00	0.00	nad	nad	nad	nad	nad	904	20
HFC143	73	49	14.9	6.3	30.4	78	23.7	1.9	5.0	0.55	4.40	0.05	0.00	0.00	nad	nad	nad	nad	nad	937	18
	74	49	15.1	6.5	30.8	75	23.2	1.4	5.4	0.76	4.59	0.05	0.00	0.00	nad	nad	nad	nad	nad	945	21
	Mean	49	15.0	6.4	30.7	76	23.4	1.7	5.0	0.56	4.39	0.08	0.00	0.00						929	20
	SD	0.6	0.12	0.10	0.31	1.7	0.26	0.26	0.35	0.190	0.205	0.052	0.000	0.000						21.7	1.5
Repeat investigation - sample removed at necropsy by aortic puncture, original sample clotted																					
	71	42	12.8	5.6	30.5	75	22.9	2.8	3.5	0.74	2.70	0.00	0.00	0.07	nad	nad	nad	nad	nad	646	22

SD Standard deviation
ctd Clotted sample
nr Not recorded

APPENDIX 9

Haematology - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no.	PCV	Hb	RBC	MCHC	MCV	MCH	Retic	WBC + Diff						Film comments					Plts	TT
		%	g/dl	$\times 10^6$/mm^3	%	fl	pg	%	Total	N	L	E	B	M	P	H	A	R	S	$\times 10^3$/mm^3	s
1♂ Air Control	1	55	16.3	7.8	29.6	71	20.9	1.8	6.2	0.93	5.21	0.06	0.00	0.00	nad	nad	nad	nad	nad	810	24
	2	53	16.3	7.2	30.8	74	22.6	0.4	5.4	0.86	4.54	0.00	0.00	0.00	nad	nad	nad	nad	nad	809	24
	3	51	15.5	7.0	30.4	73	22.1	0.7	6.7	1.74	4.89	0.07	0.00	0.00	nad	nad	nad	nad	nad	735	21
	4	51	15.3	7.2	30.0	71	21.3	3.0	6.9	1.45	5.45	0.00	0.00	0.00	1	nad	1	nad	nad	925	25
	5	53	16.3	7.4	30.8	72	22.0	1.3	8.7	2.78	5.92	0.00	0.00	0.00	nad	nad	nad	nad	nad	942	27
	Mean	53	15.9	7.3	30.3	72	21.8	1.4	6.8	1.55	5.20	0.03	0.00	0.00						844	24
	SD	1.7	0.50	0.30	0.52	1.3	0.68	1.03	1.22	0.778	0.527	0.036	0.000	0.000						87.2	2.1
2♂ 2250 ppm HFC143	11	50	15.5	6.8	31.0	74	22.8	1.1	8.4	3.95	4.45	0.00	0.00	0.00	nad	nad	nad	nad	nad	891	21
	12	52	16.0	7.2	30.8	72	22.2	1.0	6.4	1.98	4.35	0.06	0.00	0.00	nad	nad	nad	nad	nad	885	29
	13	51	15.5	7.1	30.4	72	21.8	0.2	6.4	2.30	4.03	0.00	0.00	0.06	nad	nad	1	nad	nad	885	24
	14	51	15.8	7.0	31.0	73	22.6	0.5	8.4	2.86	5.54	0.00	0.00	0.00	nad	nad	nad	nad	nad	762	24
	15	51	15.5	7.0	30.4	73	22.1	1.3	7.1	2.20	4.76	0.07	0.00	0.07	nad	nad	nad	nad	nad	772	23
	Mean	51	15.7	7.0	30.7	73	22.3	0.8	7.3	2.66	4.63	0.03	0.00	0.03						839	24
	SD	0.7	0.23	0.15	0.30	0.8	0.40	0.45	1.01	0.792	0.574	0.036	0.000	0.036						65.9	3.0
3♂ 4500 ppm HFC143	21	49	15.5	6.5	31.6	75	23.8	0.5	5.8	1.80	4.00	0.00	0.00	0.00	nad	nad	nad	nad	nad	884	23
	22	47	14.8	6.1	31.5	77	24.3	0.7	4.6	0.74	3.82	0.05	0.00	0.00	nad	nad	nad	nad	nad	98	24
	23	53	15.8	7.3	29.8	73	21.6	0.2	5.7	0.97	4.73	0.00	0.00	0.00	nad	nad	nad	nad	nad	894	23
	24	49	15.3	6.6	31.2	74	23.2	1.0	6.9	1.45	5.31	0.14	0.00	0.00	nad	nad	nad	nad	nad	777	26
	25	50	15.3	6.8	30.6	74	22.5	0.3	7.3	1.46	5.69	0.15	0.00	0.00	nad	nad	1	nad	nad	1047	24
	Mean	50	15.3	6.7	30.9	75	23.1	0.5	6.1	1.28	4.71	0.07	0.00	0.00						740	24
	SD	2.2	0.36	0.44	0.75	1.5	1.07	0.32	1.07	0.424	0.809	0.073	0.000	0.000						371.6	1.4
4♂ 9000 ppm HFC143	31	50	15.5	6.6	31.0	76	23.5	2.0	6.7	1.01	5.70	0.00	0.00	0.00	nad	nad	nad	nad	nad	735	22
	32	51	15.6	6.9	30.6	74	22.6	1.3	13.1	3.14	9.96	0.00	0.00	0.00	nad	nad	nad	nad	nad	899	23
	33	49	15.0	6.5	30.6	75	23.1	0.7	6.7	2.14	4.56	0.00	0.00	0.00	nad	nad	nad	nad	nad	807	22
	35	50	15.3	6.6	30.6	76	23.2	2.7	6.4	2.24	4.03	0.06	0.00	0.06	nad	nad	1	nad	nad	883	20
	Mean	50	15.4	6.7	30.7	75	23.1	1.7	8.2	2.13	6.06	0.02	0.00	0.02						831	22
	SD	0.8	0.26	0.17	0.20	1.0	0.37	0.87	3.25	0.873	2.690	0.030	0.000	0.030						75.5	1.2

SD Standard deviation

APPENDIX 9

(Haematology - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no.	PCV	Hb	RBC $\times 10^6$/	MCHC	MCV	MCH	Retic	WBC + Diff $\times 10^3$/mm^3						Film comments					Plts $\times 10^3$/	TT
		%	g/dl	mm^3	%	fl	pg	%	Total	N	L	E	B	M	P	H	A	R	S	mm^3	s
1♀ Air Control	41	53	15.5	7.1	29.2	75	21.8	0.4	7.4	2.00	5.33	0.00	0.00	0.07	nad	nad	nad	nad	nad	978	20
	42	48	14.9	6.5	31.0	74	22.9	0.3	8.1	1.05	6.97	0.08	0.00	0.00	nad	nad	nad	nad	nad	974	20
	43	51	15.3	7.0	30.0	73	21.9	2.1	7.8	1.79	5.77	0.16	0.00	0.08	nad	nad	nad	nad	nad	796	19
	44	46	14.7	6.2	32.0	74	23.7	4.1	12.0	3.24	8.64	0.12	0.00	0.00	1	nad	nad	nad	nad	821	19
	45	48	15.0	6.4	31.3	75	23.4	1.1	8.6	2.24	6.28	0.09	0.00	0.00	nad	nad	1	nad	nad	957	21
	Mean	49	15.1	6.6	30.7	74	22.7	1.6	8.8	2.06	6.60	0.09	0.00	0.03						905	20
	SD	2.8	0.32	0.39	1.10	0.8	0.86	1.57	1.85	0.794	1.294	0.059	0.000	0.041						89.1	0.9
2♀ 2250 ppm HFC143	51	49	14.9	6.6	30.4	74	22.6	2.1	7.5	1.50	6.00	0.00	0.00	0.00	nad	nad	nad	nad	nad	940	23
	52	49	14.9	6.8	30.4	72	21.9	3.1	8.4	2.77	5.54	0.00	0.00	0.08	1	nad	1	nad	nad	752	22
	53	49	15.0	6.7	30.6	73	22.4	1.1	7.2	0.65	6.41	0.07	0.00	0.07	nad	nad	1	nad	nad	933	21
	54	49	14.9	6.7	30.4	73	22.2	1.3	3.6	0.68	2.88	0.04	0.00	0.00	nad	nad	1	nad	nad	829	21
	55	52	15.5	7.2	29.8	72	21.5	0.9	6.7	1.34	5.16	0.20	0.00	0.00	nad	nad	1	nad	nad	963	20
	Mean	50	15.0	6.8	30.3	73	22.1	1.7	6.7	1.39	5.20	0.06	0.00	0.03						883	21
	SD	1.3	0.26	0.23	0.30	0.8	0.43	0.91	1.83	0.862	1.379	0.083	0.000	0.041						89.8	1.3
3♀ 4500 ppm HFC143	61	47	14.0	6.5	29.8	72	21.5	1.6	8.2	2.38	5.58	0.16	0.00	0.08	1	nad	1	nad	nad	1057	21
	62	46	14.5	6.0	31.5	77	24.2	0.5	6.0	0.72	5.16	0.12	0.00	0.00	nad	nad	1	nad	nad	1211	18
	63	47	14.7	6.2	31.3	76	23.7	0.4	7.5	2.18	5.25	0.08	0.00	0.00	nad	nad	1	nad	nad	929	22
	64	50	15.0	6.8	30.0	74	22.1	0.8	10.1	2.63	7.47	0.00	0.00	0.00	nad	nad	1	nad	nad	904	18
	65	49	14.7	6.6	30.0	74	22.3	1.3	10.6	4.35	6.15	0.11	0.00	0.00	nad	nad	1	nad	nad	1166	ctd
	Mean	48	14.6	6.4	30.5	75	22.8	0.9	8.5	2.45	5.92	0.09	0.00	0.02						1053	20
	SD	1.6	0.37	0.32	0.81	1.9	1.14	0.52	1.89	1.295	0.948	0.060	0.000	0.036						137.2	2.2

SD Standard deviation

ctd Clotted sample

APPENDIX 10

Haematology - individual values

Week 7 (at the end of the withdrawal period)

Group	Animal no.	PCV	Hb	RBC	MCHC	MCV	MCH	Retic	Film comments				
		%	g/dl	$\times 10^6$/ mm^3	%	fl	pg	%	P	H	A	R	S
1♂ Air Control	6	50	15.6	7.5	31.2	67	20.8	1.9	nad	nad	nad	nad	nad
	7	48	15.1	7.1	31.5	68	21.3	2.7	1	nad	1	nad	nad
	8	51	15.6	7.6	30.6	67	20.5	2.7	1	nad	1	nad	nad
	9	51	15.8	7.4	31.0	69	21.4	2.2	nad	nad	1	nad	nad
	10	48	14.7	7.2	30.6	67	20.4	2.2	1	nad	1	nad	nad
	Mean	50	15.4	7.4	31.0	68	20.9	2.3					
	SD	1.5	0.45	0.21	0.39	0.9	0.45	0.35					
2♂ 2250 ppm HFC143	16	51	16.4	7.2	32.2	71	22.8	2.1	1	nad	1	nad	nad
	17	50	15.7	7.1	31.4	70	22.1	1.3	nad	nad	nad	nad	nad
	18	48	15.4	6.9	32.1	70	22.3	1.9	1	nad	1	nad	nad
	19	49	15.1	7.0	30.8	70	21.6	2.6	1	nad	1	nad	nad
	20	48	15.4	6.6	32.1	73	23.3	1.2	1	nad	1	nad	nad
	Mean	49	15.6	7.0	31.7	71	22.4	1.8					
	SD	1.3	0.49	0.23	0.61	1.3	0.65	0.58					
3♂ 4500 ppm HFC143	26	50	15.7	7.2	31.4	69	21.8	2.2	1	nad	1	nad	nad
	27	52	15.9	7.4	30.6	70	21.5	2.8	nad	nad	1	nad	nad
	28	54	17.4	7.5	32.2	72	23.2	2.1	1	nad	nad	nad	nad
	29	53	16.7	7.5	31.5	71	22.3	2.3	nad	nad	nad	nad	nad
	30	48	15.1	6.7	31.5	72	22.5	3.3	1	nad	1	nad	nad
	Mean	51	16.2	7.3	31.4	71	22.3	2.5					
	SD	2.4	0.90	0.34	0.57	1.3	0.66	0.50					
4♂ 9000 ppm HFC143	36	47	14.7	6.6	31.3	71	22.3	3.6	1	nad	1	nad	nad
	38	48	14.9	6.8	31.0	71	21.9	1.7	1	nad	1	nad	nad
	39	47	14.6	6.7	31.1	70	21.8	2.6	1	nad	1	nad	nad
	40	50	15.7	6.9	31.4	72	22.8	3.3	1	nad	1	nad	nad
	Mean	48	15.0	6.8	31.2	71	22.2	2.8					
	SD	1.4	0.50	0.13	0.18	0.8	0.45	0.84					

SD Standard deviation

APPENDIX 10

(Haematology - continued)

Week 7 (at the end of the withdrawal period)

Group	Animal no.	PCV	Hb	RBC $\times 10^6$/ mm^3	MCHC	MCV	MCH	Retic	Film comments				
		%	g/dl		%	fl	pg	%	P	H	A	R	S
1♀	46	52	15.6	7.4	30.0	70	21.1	1.2	1	nad	1	nad	nad
Air	47	49	15.2	6.9	31.0	71	22.0	2.1	nad	nad	nad	nad	nad
Control	48	51	15.7	7.1	30.8	72	22.1	3.2	1	nad	1	nad	nad
	49	52	16.2	7.4	31.2	70	21.9	3.2	nad	nad	nad	nad	nad
	50	53	16.4	7.4	30.9	72	22.2	1.5	nad	nad	nad	nad	nad
	Mean	51	15.8	7.2	30.8	71	21.9	2.2					
	SD	1.5	0.48	0.23	0.46	1.0	0.44	0.93					
2♀	56	52	16.2	7.1	31.2	73	22.8	2.1	nad	nad	nad	nad	nad
2250 ppm	57	52	15.7	7.3	30.2	71	21.5	1.6	1	nad	1	nad	nad
HFC 143	58	53	15.9	7.5	30.0	71	21.2	1.8	1	nad	1	nad	nad
	59	49	15.5	6.7	31.6	73	23.1	2.0	1	nad	1	nad	nad
	60	53	16.2	7.4	30.6	72	21.9	1.7	nad	nad	nad	nad	nad
	Mean	52	15.9	7.2	30.7	72	22.1	1.8					
	SD	1.6	0.31	0.32	0.67	1.0	0.82	0.21					
3♀	66	52	15.9	7.3	30.6	71	21.8	0.9	nad	nad	nad	nad	nad
4500 ppm	67	46	14.6	6.3	31.7	73	23.2	2.5	1	nad	1	nad	nad
HFC 143	68	49	15.5	6.6	31.6	74	23.5	1.6	1	nad	1	nad	nad
	69	51	15.9	6.9	31.2	74	23.0	1.3	1	nad	1	nad	nad
	70	50	15.7	6.9	31.4	72	22.8	3.8	1	nad	1	nad	nad
	Mean	50	15.5	6.8	31.3	73	22.9	2.0					
	SD	2.3	0.54	0.37	0.44	1.3	0.65	1.16					

SD Standard deviation

APPENDIX 11

Biochemistry - individual values

Week 3 (5 January 1994)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
4♀	71	114	5.9	3.0	2.9	17	0.7	148	24	60	<1	137	0.2	145	4.2	5.3	4.2	102	67
9000 ppm	72	124	6.8	3.2	3.6	20	0.6	221	23	43	1	115	0.2	144	3.8	5.8	4.4	102	89
HFC 143	73	136	6.9	3.2	3.7	18	0.6	203	19	43	<1	122	0.2	141	3.3	5.8	4.0	99	115
	74	111	6.0	3.0	3.0	16	0.6	331	20	50	2	89	0.2	141	3.6	5.4	3.8	98	83
	Mean	121	6.4	3.1	3.3	18	0.6	226	22	49	<2	116	0.2	143	3.7	5.6	4.1	100	89
	SD	11.3	0.52	0.12	0.41	1.7	0.05	76.7	2.4	8.0		20.1	0.00	2.1	0.38	0.26	0.26	2.1	20.0

SD Standard deviation

APPENDIX 12

Biochemistry - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
1♂ Air Control	1	114	6.5	2.9	3.6	14	0.5	355	31	53	1	150	0.2	143	3.4	5.1	4.3	95	67
	2	105	6.5	2.8	3.7	14	0.5	266	27	48	<1	237	0.1	144	3.2	5.1	4.9	98	60
	3	116	6.6	3.1	3.5	14	0.5	292	34	68	<1	269	0.1	144	3.0	5.4	4.1	99	107
	4	121	6.0	2.9	3.1	12	0.4	312	31	53	<1	89	0.1	143	3.4	5.1	3.9	98	61
	5	100	6.8	3.0	3.8	14	0.5	461	37	65	<1	188	0.1	143	3.2	5.4	4.1	99	46
	Mean	111	6.5	2.9	3.5	14	0.5	337	32	57	<1	187	0.1	143	3.2	5.2	4.3	98	68
	SD	8.5	0.29	0.11	0.27	0.9	0.04	76.5	3.7	8.6		71.1	0.04	0.5	0.17	0.16	0.38	1.6	23.0
2♂ 2250 ppm HFC143	11	127	6.7	2.9	3.8	15	0.6	447	37	56	<1	119	0.2	143	3.4	5.2	4.4	98	73
	12	123	6.6	2.9	3.7	12	0.5	268	22	48	1	107	0.1	145	3.2	5.2	4.1	102	53
	13	107	6.4	3.1	3.3	13	0.4	426	31	54	<1	63	0.1	145	3.2	5.2	3.9	100	64
	14	103	5.8	2.8	3.0	12	0.5	261	30	56	<1	201	0.2	145	3.4	5.3	4.1	101	53
	15	122	6.2	2.9	3.3	15	0.5	331	37	62	<1	122	0.1	144	3.1	5.2	4.5	99	101
	Mean	116	6.3	2.9	3.4	13	0.5	347	31	55	<1	122	0.1	144	3.3	5.2	4.2	100	69
	SD	10.7	0.36	0.11	0.33	1.5	0.07	86.8	6.2	5.0		49.9	0.05	0.9	0.13	0.04	0.24	1.6	19.9
3♂ 4500 ppm HFC143	21	102	6.2	2.9	3.3	13	0.6	305	30	52	<1	133	0.1	145	3.4	5.3	4.9	100	54
	22	125	6.0	2.8	3.2	15	0.6	303	26	47	<1	51	0.2	144	3.2	5.0	5.1	100	57
	23	113	6.0	3.0	3.0	15	0.5	328	30	57	<1	75	0.1	145	3.5	5.1	4.5	100	66
	24	128	6.4	2.9	3.5	13	0.5	263	22	49	<1	254	0.1	143	3.1	5.2	3.5	99	63
	25	101	5.8	2.7	3.1	14	0.6	295	33	63	<1	65	0.1	145	3.0	5.1	4.4	102	41
	Mean	114	6.1	2.9	3.2	14	0.6	299	28	54	<1	116	0.1	144	3.2	5.1	4.5	100	56
	SD	12.6	0.23	0.11	0.19	1.0	0.05	23.5	4.3	6.5		83.4	0.04	0.9	0.21	0.11	0.62	1.1	9.7
4♂ 9000 ppm HFC143	31	108	6.5	2.8	3.7	13	0.5	279	27	54	<1	121	0.1	144	3.2	5.3	4.5	98	122
	32	113	6.5	3.0	3.5	13	0.6	346	32	54	<1	135	0.2	143	3.0	5.3	3.9	97	53
	33	104	6.1	2.9	3.2	13	0.5	318	26	55	<1	107	0.1	147	3.3	5.1	4.9	102	70
	35	127	6.0	2.8	3.2	14	0.4	284	32	83	<1	91	0.1	146	3.8	5.3	4.9	102	102
	Mean	113	6.3	2.9	3.4	13	0.5	307	29	62	<1	114	0.1	145	3.3	5.3	4.6	100	87
	SD	10.0	0.26	0.10	0.24	0.5	0.08	31.4	3.2	14.3		18.9	0.05	1.8	0.34	0.10	0.47	2.6	31.1

SD Standard deviation

APPENDIX 12

(Biochemistry - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no.	Glucose mg/dl	Protein g/dl			Urea Nitr mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
			Total	Alb	Glob														
1♀	41	119	6.4	2.9	3.5	15	0.6	241	27	59	<1	249	0.1	145	3.8	5.2	4.6	104	69
Air	42	106	6.0	3.2	2.8	17	0.5	168	27	47	<1	77	0.1	142	3.6	5.3	4.0	100	77
Control	43	108	6.8	3.2	3.6	21	0.6	296	29	57	<1	157	0.2	143	3.9	5.6	4.4	99	76
	44	117	7.6	3.3	4.3	15	0.6	151	24	41	<1	74	0.1	140	3.2	5.2	3.5	99	97
	45	113	6.8	3.1	3.7	17	0.6	306	25	45	<1	106	0.2	141	3.6	5.3	3.9	99	71
	Mean	113	6.7	3.1	3.6	17	0.6	232	26	50	<1	133	0.1	142	3.6	5.3	4.1	100	78
	SD	5.6	0.59	0.15	0.54	2.4	0.04	71.3	1.9	7.8		73.1	0.05	1.9	0.27	0.16	0.43	2.2	11.1
2♀	51	107	6.6	3.2	3.4	19	0.6	205	26	54	<1	179	0.1	144	3.2	5.5	4.3	101	54
2250 ppm	52	119	6.8	3.3	3.5	19	0.6	167	29	54	<1	95	0.2	139	3.1	5.4	3.6	97	55
HFC143	53	111	6.3	3.0	3.3	18	0.5	216	26	54	<1	75	0.2	142	3.0	5.3	3.9	98	50
	54	116	6.6	3.2	3.4	14	0.6	193	22	54	<1	224	0.1	144	3.4	5.4	4.0	102	72
	55	124	6.7	3.0	3.7	17	0.6	207	28	62	<1	179	0.1	140	3.2	5.2	3.6	97	112
	Mean	115	6.6	3.1	3.5	17	0.6	198	26	56	<1	150	0.1	142	3.2	5.4	3.9	99	69
	SD	6.7	0.19	0.13	0.15	2.1	0.04	19.0	2.7	3.6		62.9	0.05	2.3	0.15	0.11	0.29	2.3	25.7
3♀	61	124	6.3	3.0	3.3	13	0.5	202	29	54	<1	85	0.2	142	2.9	5.1	3.4	99	77
4500 ppm	62	105	6.4	3.1	3.3	17	0.6	249	24	43	<1	55	0.2	143	3.3	5.3	4.5	101	64
HFC143	63	128	6.2	2.9	3.3	19	0.5	176	16	41	<1	45	0.1	141	3.3	5.3	4.4	99	66
	64	126	6.3	3.2	3.1	14	0.5	271	26	52	<1	296	0.1	143	2.8	5.1	3.7	98	77
	65	112	6.6	3.1	3.5	18	0.6	197	24	45	<1	98	0.2	143	3.1	5.2	4.1	99	82
	Mean	119	6.4	3.1	3.3	16	0.5	219	24	47	<1	116	0.2	142	3.1	5.2	4.0	99	73
	SD	10.0	0.15	0.11	0.14	2.6	0.05	39.5	4.8	5.7		103.0	0.05	0.9	0.23	0.10	0.47	1.1	7.8

SD Standard deviation

APPENDIX 13

Urinalysis - individual values

Week 3 (5 January 1994)

Group	Animal no.	Appr	Colour	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments
4♀	71	N	LS	1.8	6.1	1072	70	0	0	0	0	0	0
9000 ppm	72	N	LS	5.0	5.6	1032	64	0	0	0	0	0	0
HFC143	73	N	LS	5.6	5.6	1026	54	0	0	0	0	0	0
	74	N	LS	2.0	5.4	1034	76	0	0	0	0	0	0
	Mean			3.6	5.7	1041	66						
	SD			1.98	0.30	20.9	9.4						

SD Standard deviation
LS Light straw

APPENDIX 14

Urinalysis - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no.	Appr	Colour	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments
1♂ Air Control	1	N	LS	7.2	6.6	1027	130	+	0	+	0	0	0
	2	N	LS	9.8	6.9	1030	115	+	0	++	0	0	0
	3	N	LS	6.8	6.8	1033	117	+	0	tr	0	0	0
	4	N	LS	5.8	7.0	1031	142	+	0	+	0	0	0
	5	N	MS	4.4	6.8	1048	164	+	0	++	0	0	0
	Mean			6.8	6.8	1034	134						
	SD			1.99	0.15	8.2	20.2						
2♂ 2250 ppm HFC143	11	N	LS	4.6	6.2	1044	165	+	0	0	0	0	0
	12	N	LS	6.2	6.7	1033	148	+	0	tr	0	0	0
	13	N	LS	4.6	6.5	1045	145	+	0	tr	0	0	0
	14	N	LS	7.2	6.5	1036	162	+	0	tr	0	0	0
	15	N	LS	5.0	6.6	1026	143	+	0	++	0	0	0
	Mean			5.5	6.5	1037	153						
	SD			1.15	0.19	7.9	10.2						
3♂ 4500 ppm HFC143	21	N	LS	5.2	6.5	1029	144	+	0	++	0	0	0
	22	N	VLS	7.2	6.5	1032	104	tr	0	+	0	0	0
	23	N	MS	3.8	6.1	1022	133	+	0	++	0	0	0
	24	N	LS	6.0	6.2	1040	165	+	0	+	0	0	0
	25	N	LS	4.6	6.3	1047	151	+	0	+	0	0	0
	Mean			5.4	6.3	1034	139						
	SD			1.31	0.18	9.7	22.9						
4♂ 9000 ppm HFC143	31	N	LS	3.4	6.4	1040	122	+	0	+	0	0	0
	32	N	LS	6.6	6.2	1041	161	+	0	+	0	0	0
	33	N	LS	5.2	6.0	1035	147	+	0	+	0	0	0
	35	N	LS	6.2	6.2	1038	125	+	0	tr	0	0	0
	Mean			5.4	6.2	1039	139						
	SD			1.43	0.16	2.6	18.6						

SD Standard deviation
VLS Very light straw
LS Light straw
MS Medium straw

APPENDIX 14

(Urinalysis - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no.	Appr	Colour	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments
1♀ Air Control	41	N	LS	3.2	6.7	1046	97	+	0	0	0	0	0
	42	N	LS	2.4	6.3	1046	94	0	0	tr	0	0	0
	43	N	LS	4.0	6.3	1042	71	0	0	tr	0	0	0
	44	N	LS	6.2	6.6	1032	61	0	0	tr	0	0	0
	45	N	LS	5.2	6.4	1036	57	0	0	tr	0	0	0
	Mean			4.2	6.5	1040	76						
	SD			1.52	0.18	6.2	18.5						
2♀ 2250 ppm HFC143	51	N	LS	3.2	6.2	1042	79	0	0	tr	0	0	0
	52	N	LS	4.0	6.2	1037	56	0	0	tr	0	0	0
	53	C	LS	4.2	6.6	1040	80	0	0	tr	0	0	0
	54	N	VLS	4.4	6.5	1030	52	0	0	tr	0	0	0
	55	N	LS	5.4	6.4	1038	59	0	0	0	0	0	0
	Mean			4.2	6.4	1037	65						
	SD			0.79	0.18	4.6	13.3						
3♀ 4500 ppm HFC143	61	N	LS	5.8	6.0	1038	61	0	0	0	0	0	0
	62	N	LS	3.8	6.1	1037	73	tr	0	0	0	0	0
	63	N	LS	3.2	6.0	1036	59	0	0	0	0	0	0
	64	N	VLS	5.6	5.9	1020	54	0	0	0	0	0	0
	65	N	VLS	9.2	5.9	1025	41	0	0	0	0	0	0
	Mean			5.5	6.0	1031	58						
	SD			2.34	0.08	8.2	11.6						

SD Standard deviation
VLS Very light straw
LS Light straw

APPENDIX 15

Urinalysis - individual values

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Volume ml	pH
1♂ Air Control	6	6.3	6.7
	7	11.0	6.6
	8	8.3	6.9
	9	7.2	6.3
	10	10.2	6.6
	Mean	8.6	6.6
	SD	1.98	0.22
2♂ 2250 ppm HFC 143	16	7.4	6.8
	17	8.3	6.9
	18	10.2	6.8
	19	7.3	6.8
	20	7.4	6.7
	Mean	8.1	6.8
	SD	1.23	0.07
3♂ 4500 ppm HFC 143	26	11.0	6.9
	27	3.2	6.1
	28	9.1	6.7
	29	5.0	6.6
	30	8.4	6.7
	Mean	7.3	6.6
	SD	3.17	0.30
4♂ 9000 ppm HFC 143	36	11.0	7.2
	38	8.2	6.7
	39	8.2	6.6
	40	3.1	6.6
	Mean	7.6	6.8
	SD	3.29	0.29

SD Standard deviation

APPENDIX 15

(Urinalysis - continued)

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Volume ml	pH
1♀ Air Control	46	4.3	6.7
	47	5.3	6.8
	48	4.2	6.8
	49	5.2	6.2
	50	8.4	6.9
	Mean	5.5	6.7
	SD	1.71	0.28
2♀ 2250 ppm HFC 143	56	5.0	6.4
	57	4.3	6.6
	58	6.2	7.1
	59	4.0	6.2
	60	8.1	6.5
	Mean	5.5	6.6
	SD	1.67	0.34
3♀ 4500 ppm HFC 143	66	3.2	6.8
	67	5.4	6.3
	68	4.4	6.1
	69	3.3	6.6
	70	4.1	6.2
	Mean	4.1	6.4
	SD	0.90	0.29

SD Standard deviation

APPENDIX 16

Urinary inorganic fluoride - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no./sex	Urine volume (ml)	Fluoride concentration (µg/ml)	Total fluoride (µg)
1 Air Control	1♂	7.2	1.8	12.96
	2♂	9.8	1.5	14.70
	3♂	6.8	1.6	10.88
	4♂	5.8	1.6	9.28
	5♂	4.4	3.2	14.08
	6♂	6.0	1.8	10.80
	7♂	10.8	1.2(1.1)	12.96
	8♂	10.2	1.3	13.26
	9♂	8.2	1.6	13.12
	10♂	10.2	1.3	13.26
	Mean	7.94	1.69	12.53
	SD	2.227	0.569	1.672
2 2250 ppm HFC 143	11♂	4.6	3.8	17.48
	12♂	6.2	3.5	21.70
	13♂	4.6	4.4	20.24
	14♂	7.2	2.4	17.28
	15♂	5.0	4.2	21.00
	16♂	7.0	3.1	21.70
	17♂	6.2	3.2	19.84
	18♂	7.2	2.4	17.28
	19♂	6.2	3.5	21.70
	20♂	6.6	3.3	21.78
	Mean	6.08	3.38**	20.00**
	SD	1.012	0.663	1.946
3 4500 ppm HFC 143	21♂	5.2	5.0	26.00
	22♂	7.2	4.2	30.24
	23♂	3.8	4.0	15.20
	24♂	6.0	4.5	27.00
	25♂	4.6	4.0	18.40
	26♂	5.8	4.0	23.20
	27♂	4.2	5.3	22.26
	28♂	7.2	3.3	23.76
	29♂	6.0	3.7	22.20
	30♂	6.4	3.7(3.8)	23.68
	Mean	5.64	4.17**	23.19**
	SD	1.177	0.611	4.234
4 9000 ppm HFC 143	31♂	3.4	4.2	14.28
	32♂	6.6	3.6	23.76
	33♂	5.2	3.7	19.24
	34♂a			
	35♂	6.2	3.6	22.32
	36♂	9.4	3.1(2.7,2.8)	29.14
	37♂a			
	38♂	12.0	2.3	27.60
	39♂	5.8	5.1	29.58
	40♂	6.4	3.7	23.68
	Mean	6.88	3.66**	23.70**
	SD	2.657	0.805	5.209

a Unscheduled death - no urine available
( ) Duplicated analysis
** P < 0.01 compared with control data using Williams' test

APPENDIX 16

(Urinary inorganic fluoride - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no./sex	Urine volume (ml)	Fluoride concentration (μg/ml)	Total fluoride (μg)
1 Air Control*	41♀	3.2	2.3	7.36
	42♀	2.4	2.2	5.28
	43♀	4.0	1.7	6.80
	44♀	6.2	1.4	8.68
	45♀	5.2	1.7	8.84
	46♀	5.8	1.5	8.70
	47♀	3.8	1.6	6.08
	48♀	6.2	1.4	8.68
	49♀	2.8	2.0	5.60
	50♀	9.8	1.2(1.2)	11.76
	Mean	4.94	1.70	7.78
	SD	2.207	0.362	1.957
2 2250 ppm HFC 143	51♀	3.2	2.8	8.96
	52♀	4.0	2.1	8.40
	53♀	4.2	2.9	12.18
	54♀	4.4	2.7	11.88
	55♀	5.4	2.6	14.04
	56♀	3.4	4.2	14.28
	57♀	3.6	2.8	10.08
	58♀	7.8	2.1	16.38
	59♀	2.6	2.6	6.76
	60♀	7.0	2.1	14.70
	Mean	4.56	2.69**	11.77**
	SD	1.686	0.615	3.139
3 4500 ppm HFC 143	61♀	5.8	3.2	18.56
	62♀	3.8	4.0	15.20
	63♀	3.2	4.5	14.40
	64♀	5.6	2.7	15.12
	65♀	9.2	1.8(1.8)	16.56
	66♀	3.4	3.0	10.20
	67♀	5.4	3.3	17.82
	68♀	4.6	3.1	14.26
	69♀	4.4	4.4	19.36
	70♀	3.0	4.9	14.70
	Mean	4.84	3.49**	15.62**
	SD	1.835	0.948	2.632
4 9000 ppm HFC 143	71♀#	1.8	7.0	12.60
	72♀#	5.0	3.1	15.50
	73♀#	5.6	3.4	19.04
	74♀#	2.0	6.0	12.00
	75♀a			
	76♀a			
	77♀a			
	78♀a			
	79♀a			
	80♀a			
	Mean	3.6	4.88	14.79
	SD	1.98	1.924	3.222

a Unscheduled death - no urine available
\# Samples removed 5 January 1994, prior to sacrifice (Week 3)
( ) Duplicated analysis
** P <0.01 compared with control data using Williams' test

APPENDIX 17

Urinary inorganic fluoride - individual values

Week 7 (at the end of the withdrawal period)

Group	Animal no./sex	Urine volume (ml)	Fluoride concentration (μg/ml)	Total fluoride (μg)
1 Air Control	6♂	6.3	1.7	10.71
	7♂	11.0	1.1	12.10
	8♂	8.3	1.8(1.7)	14.94
	9♂	7.2	1.6	11.52
	10♂	10.2	1.1	11.22
	Mean	8.6	1.46	12.10
	SD	1.98	0.336	1.667
2 2250 ppm HFC 143	16♂	7.4	1.6	11.84
	17♂	8.3	1.6	13.28
	18♂	10.2	1.4	14.28
	19♂	7.3	1.6	11.68
	20♂	7.4	1.7	12.58
	Mean	8.1	1.58	12.73
	SD	1.23	0.110	1.075
3 4500 ppm HFC 143	26♂	11.0	1.5	16.50
	27♂	3.2	3.4	10.88
	28♂	9.1	1.7	15.47
	29♂	5.0	2.6	13.00
	30♂	8.4	2.0	16.80
	Mean	7.3	2.24*	14.53
	SD	3.17	0.770	2.529
4 9000 ppm HFC 143	36♂	11.0	2.0	22.00
	37♂a			
	38♂	8.2	1.7	13.94
	39♂	8.2	1.7	13.94
	40♂	3.1	3.6	11.16
	Mean	7.6	2.25*	15.26*
	SD	3.29	0.911	4.681

a Unscheduled death - no urine available

( ) Duplicated analysis

* $P < 0.05$ compared with control data using Williams' test

APPENDIX 17

(Urinary inorganic fluoride - continued)

Week 7 (at the end of the withdrawal period)

Group	Animal no./sex	Urine volume (ml)	Fluoride concentration (μg/ml)	Total fluoride (μg)
1 Air Control	46♀	4.3	1.1	4.73
	47♀	5.3	1.1	5.83
	48♀	4.2	1.4	5.88
	49♀	5.2	1.4	7.28
	50♀	8.4	1.1(1.2)	9.24
	Mean	5.5	1.22	6.59
	SD	1.71	0.164	1.735
2 2250 ppm HFC 143	56♀	5.0	1.4	7.00
	57♀	4.3	1.4	6.02
	58♀	6.2	1.5	9.30
	59♀	4.0	1.7	6.80
	60♀	8.1	1.4	11.34
	Mean	5.5	1.48	8.09
	SD	1.67	0.130	2.189
3 4500 ppm HFC 143	66♀	3.2	2.1	6.72
	67♀	5.4	1.6(1.6)	8.64
	68♀	4.4	2.0	8.80
	69♀	3.3	2.4	7.92
	70♀	4.1	1.7	6.97
	Mean	4.1	1.96**	7.81
	SD	0.90	0.321	0.945

( ) Duplicated analysis

** $P < 0.01$ compared with control data using Williams' test

APPENDIX 18

Bone myelograms - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no.	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♂ Air Control	1	27.0	18.0	55.0	1.50
	2	31.5	41.5	27.0	0.76
	3	37.0	27.5	35.5	1.35
	4	44.5	34.0	21.5	1.31
	5	48.5	21.0	30.5	2.31
	Mean	37.7	28.4	33.9	1.45
	SD	8.89	9.57	12.85	0.559
2♂ 2250 ppm HFC 143	11	44.5	18.0	37.5	2.47
	12	37.5	19.0	43.5	1.97
	13	49.5	23.0	27.5	2.15
	14	40.5	20.0	39.5	2.03
	15	38.0	32.5	29.5	1.17
	Mean	42.0	22.5	35.5	1.96
	SD	5.02	5.89	6.78	0.481
3♂ 4500 ppm HFC 143	21	49.0	27.5	23.5	1.78
	22	30.5	19.5	50.0	1.56
	23	33.0	20.0	47.0	1.65
	24	30.5	16.0	53.5	1.91
	25	35.5	16.0	48.5	2.22
	Mean	35.7	19.8	44.5	1.82
	SD	7.72	4.70	11.98	0.258
4♂ 9000 ppm HFC 143	31	30.0	40.5	29.5	0.74
	32	31.0	27.5	41.5	1.13
	33	36.5	23.0	40.5	1.59
	35	43.0	16.5	40.5	2.61
	Mean	35.1	26.9	38.0	1.52
	SD	5.98	10.14	5.69	0.807

SD Standard deviation

APPENDIX 18

(Bone myelograms - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no.	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♀ Air Control	41	37.5	24.5	38.0	1.53
	42	32.5	23.5	44.0	1.38
	43	34.5	31.5	34.0	1.10
	44	40.5	23.5	36.0	1.72
	45	33.5	17.0	49.5	1.97
	Mean	35.7	24.0	40.3	1.54
	SD	3.27	5.15	6.36	0.330
2♀ 2250 ppm HFC 143	51	39.0	35.0	26.0	1.11
	52	39.5	27.0	33.5	1.46
	53	35.0	28.0	37.0	1.25
	54	45.0	25.5	29.5	1.76
	55	35.5	26.0	38.5	1.37
	Mean	38.8	28.3	32.9	1.39
	SD	4.01	3.87	5.19	0.245
3♀ 4500 ppm HFC 143	61	30.0	27.5	42.5	1.09
	62	28.5	28.0	43.5	1.02
	63	31.5	20.0	48.5	1.58
	64	30.5	25.5	44.0	1.20
	65	26.0	32.0	42.0	0.81
	Mean	29.3	26.6	44.1	1.14
	SD	2.14	4.38	2.58	0.284
Rats killed during Week 3					
4♀ 9000 ppm HFC 143	71	25.5	33.0	41.5	0.77
	72	37.5	19.0	43.5	1.97
	73	47.5	31.0	21.5	1.53
	74	46.5	20.5	33.0	2.27
	Mean	39.3	25.9	34.9	1.64
	SD	10.21	7.15	10.01	0.652

SD Standard deviation

APPENDIX 19

Bone myelograms - individual values

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♂ Air Control	6	39.0	26.0	35.0	1.50
	7	41.0	32.0	27.0	1.28
	8	53.0	24.5	22.5	2.16
	9	33.5	30.0	36.5	1.12
	10	52.0	23.0	25.0	2.26
	Mean	43.7	27.1	29.2	1.66
	SD	8.50	3.78	6.21	0.518
2♂ 2250 ppm HFC 143	16	44.5	19.5	36.0	2.28
	17	50.5	17.0	32.5	2.97
	18	46.5	27.0	26.5	1.72
	19	27.5	29.0	43.5	0.95
	20	41.0	21.0	38.0	1.95
	Mean	42.0	22.7	35.3	1.97
	SD	8.80	5.09	6.33	0.742
3♂ 4500 ppm HFC 143	26	48.5	23.0	28.5	2.11
	27	36.5	31.0	32.5	1.18
	28	35.5	30.0	34.5	1.18
	29	43.0	27.5	29.5	1.56
	30	37.0	18.5	44.5	2.00
	Mean	40.1	26.0	33.9	1.61
	SD	5.54	5.21	6.39	0.440
4♂ 9000 ppm HFC 143	36	32.5	30.5	37.0	1.07
	38	45.0	25.0	30.0	1.80
	39	45.0	32.5	22.5	1.38
	40	47.5	30.5	22.0	1.56
	Mean	42.5	29.6	27.9	1.45
	SD	6.77	3.22	7.10	0.308

SD Standard deviation

APPENDIX 19

(Bone myelograms - continued)

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Total Myelo cells	Total Eryt-hroid	Others	M : E Ratio
1♀ Air Control	46	41.5	22.5	36.0	1.84
	47	39.5	27.5	33.0	1.44
	48	46.0	23.0	31.0	2.00
	49	38.0	32.5	29.5	1.17
	50	38.0	32.0	30.0	1.19
	Mean	40.6	27.5	31.9	1.53
	SD	3.34	4.76	2.66	0.378
2♀ 2250 ppm HFC 143	56	41.0	28.0	31.0	1.46
	57	31.0	41.5	27.5	0.75
	58	46.0	30.5	23.5	1.51
	59	31.0	23.5	45.5	1.32
	60	41.0	20.5	38.5	2.00
	Mean	38.0	28.8	33.2	1.41
	SD	6.71	8.09	8.81	0.448
3♀ 4500 ppm HFC 143	66	39.0	27.5	33.5	1.42
	67	34.5	26.5	39.0	1.30
	68	48.5	26.0	25.5	1.87
	69	37.0	32.5	30.5	1.14
	70	44.5	27.5	28.0	1.62
	Mean	40.7	28.0	31.3	1.47
	SD	5.71	2.60	5.23	0.284

SD Standard deviation

APPENDIX 20

Organ weights - individual values

Week 3 (5 January 1994)

Group	Animal no.	Body wt	Lungs	Liver	Kidneys	Adrenals
		g	g	g	g	mg
4♀ 9000 ppm HFC 143	71	200	0.92	6.9	1.53	59.2
	72	216	1.09	8.1	1.65	59.1
	73	223	1.11	9.0	1.64	63.6
	74	207	1.32	8.4	1.56	58.1
	Mean	212	1.11	8.1	1.60	60.0
	SD	10.0	0.166	0.92	0.058	2.45

SD Standard deviation

APPENDIX 21

Organ weights - individual values

Week 5 (at the end of the period of exposures)

Group	Animal no.	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Epididymides L g	Epididymides R g
1♂ Air Control	1	318	1.18	10.5	2.27	44.9	1.59	1.61	0.512	0.462
	2	367	1.40	14.3	2.93	49.7	1.65	1.66	0.426	0.451
	3	362	1.27	13.3	2.39	53.6	1.55	1.58	0.426	0.410
	4	359	1.27	14.7	2.49	50.9	1.48	1.58	0.391	0.393
	5	343	1.37	12.5	2.49	49.4	1.41	1.44	0.435	0.458
	Mean	350	1.30	13.1	2.51	49.7	1.54	1.57	0.438	0.435
	SD	19.7	0.090	1.66	0.251	3.15	0.095	0.082	0.0447	0.0312
2♂ 2250 ppm HFC143	11	327	1.26	11.2	2.19	45.0	0.70	0.68	0.353	0.346
	12	346	1.19	9.3	2.17	52.4	0.86	0.79	0.283	0.292
	13	348	1.32	11.9	2.73	48.1	1.24	1.24	0.303	0.343
	14	378	1.50	13.0	2.57	56.7	1.34	1.35	0.428	0.472
	15	366	1.41	10.9	2.50	59.7	1.11	0.97	0.394	0.391
	Mean	353	1.34	11.3	2.43	52.4	1.05	1.01	0.352	0.369
	SD	19.6	0.122	1.39	0.244	6.02	0.268	0.285	0.0606	0.0675
3♂ 4500 ppm HFC143	21	352	1.17	9.2	2.26	49.0	1.10	1.13	0.394	0.386
	22	305	1.15	9.9	2.22	53.5	0.56	0.64	0.378	0.318
	23	325	1.25	9.6	2.47	43.4	0.73	0.71	0.270	0.279
	24	367	1.40	12.0	2.42	52.1	0.62	0.64	0.288	0.304
	25	364	1.20	11.9	2.54	66.9	1.01	0.95	0.434	0.392
	Mean	343	1.23	10.5	2.38	53.0	0.80	0.81	0.353	0.336
	SD	26.7	0.099	1.34	0.139	8.70	0.238	0.220	0.0707	0.0506
4♂ 9000 ppm HFC143	31	324	1.29	9.9	2.36	45.7	0.55	0.54	0.274	0.259
	32	360	1.34	12.6	2.57	55.3	0.82	0.80	0.289	0.306
	33	289	1.24	9.3	2.09	53.8	0.55	0.52	0.278	0.247
	35	315	1.29	9.9	2.18	68.2	0.59	0.58	0.233	0.230
	Mean	322	1.29	10.4	2.30	55.8	0.63	0.61	0.269	0.261
	SD	29.6	0.038	1.49	0.212	9.31	0.131	0.129	0.0245	0.0326

SD Standard deviation

APPENDIX 21

(Organ weights - continued)

Week 5 (at the end of the period of exposures)

Group	Animal no.	Body wt	Lungs	Liver	Kidneys	Adrenals
		g	g	g	g	mg
1♀ Air Control	41	229	1.05	7.3	1.73	72.4
	42	220	1.18	7.4	1.59	61.8
	43	232	1.26	7.6	1.50	60.2
	44	248	1.01	8.8	1.70	67.8
	45	213	0.95	6.0	1.45	59.6
	Mean	228	1.09	7.4	1.59	64.4
	SD	13.3	0.128	1.02	0.121	5.55
2♀ 2250 ppm HFC143	51	236	1.05	9.0	1.67	67.8
	52	226	1.07	8.8	1.55	67.9
	53	237	1.10	8.3	1.77	61.8
	54	212	1.03	8.0	1.73	58.0
	55	230	1.00	7.1	1.99	66.9
	Mean	228	1.05	8.2	1.74	64.5
	SD	9.9	0.040	0.77	0.163	4.41
3♀ 4500 ppm HFC143	61	244	1.07	9.4	2.10	63.0
	62	209	0.97	7.7	1.62	60.4
	63	233	1.17	8.1	1.64	70.2
	64	247	1.16	9.5	1.78	73.1
	65	235	1.01	8.3	1.93	66.6
	Mean	234	1.07	8.6	1.81	66.7
	SD	15.1	0.090	0.80	0.205	5.16

SD Standard deviation

APPENDIX 22

Organ weights - individual values

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Body wt g	Lungs g	Liver g	Kidneys g	Adrenals mg	Testes L g	Testes R g	Epididymides L g	Epididymides R g
1♂ Air Control	6	335	1.38	8.9	2.22	59.6	1.48	1.54	0.470	0.455
	7	397	1.29	11.2	2.46	47.1	1.76	1.75	0.533	0.507
	8	373	1.42	9.6	2.13	53.0	1.39	1.39	0.497	0.483
	9	390	1.38	10.1	2.26	56.1	1.54	1.57	0.495	0.547
	10	418	1.38	11.8	2.71	47.3	1.72	1.76	0.527	0.556
	Mean	383	1.37	10.3	2.35	52.6	1.58	1.60	0.504	0.510
	SD	31.4	0.046	1.20	0.230	5.47	0.158	0.155	0.0258	0.0426
2♂ 2250 ppm HFC 143	16	383	1.37	10.3	2.30	55.3	1.14	1.14	0.379	0.396
	17	399	1.66	10.5	2.43	67.3	0.97	0.98	0.365	0.378
	18	414	1.54	11.7	2.56	56.2	1.04	0.96	0.424	0.372
	19	353	1.32	9.0	2.21	52.4	1.06	1.06	0.402	0.380
	20	400	1.63	11.0	2.54	63.2	0.68	0.68	0.383	0.427
	Mean	390	1.50	10.5	2.41	58.9	0.98	0.96	0.391	0.391
	SD	23.4	0.151	1.00	0.153	6.16	0.177	0.173	0.0229	0.0222
3♂ 4500 ppm HFC 143	26	433	1.38	12.4	2.55	70.9	0.69	0.63	0.323	0.287
	27	367	1.27	10.6	2.57	47.0	0.70	0.78	0.288	0.263
	28	379	1.41	10.8	2.18	66.1	0.59	0.63	0.307	0.285
	29	370	1.34	10.2	2.13	74.2	0.63	0.71	0.319	0.382
	30	437	1.37	12.5	2.30	52.4	0.54	0.58	0.264	0.308
	Mean	397	1.35	11.3	2.35	62.1	0.63	0.66	0.300	0.305
	SD	35.0	0.054	1.08	0.206	11.85	0.066	0.078	0.0244	0.0459
4♂ 9000 ppm HFC 143	36	330	1.30	8.5	2.00	68.4	0.50	0.53	0.281	0.298
	38	316	1.29	8.0	2.10	61.6	0.64	0.62	0.228	0.266
	39	382	1.47	9.7	2.42	50.6	0.62	0.63	0.302	0.327
	40	334	1.33	9.4	2.27	64.4	0.72	0.59	0.261	0.279
	Mean	340	1.35	8.9	2.20	61.3	0.62	0.59	0.268	0.293
	SD	29.1	0.081	0.80	0.187	7.63	0.090	0.045	0.0315	0.0265

SD Standard deviation

APPENDIX 22

(Organ weights - continued)

Week 7 (at the end of the withdrawal period)

Group	Animal no.	Body wt	Lungs	Liver	Kidneys	Adrenals
		g	g	g	g	mg
1♀ Air Control	46	294	1.23	9.1	2.01	74.6
	47	262	1.17	7.8	1.69	75.6
	48	286	1.16	8.0	1.53	62.2
	49	228	1.00	6.7	1.41	63.2
	50	275	1.13	7.8	1.62	69.7
	Mean	269	1.14	7.9	1.65	69.1
	SD	25.6	0.088	0.85	0.228	6.23
2♀ 2250 ppm HFC 143	56	260	1.23	8.5	1.69	74.6
	57	248	1.06	8.5	1.69	59.8
	58	301	1.28	11.0	2.03	86.0
	59	249	1.14	8.0	1.52	63.3
	60	286	1.21	9.4	1.88	77.0
	Mean	269	1.18	9.1	1.76	72.1
	SD	23.5	0.087	1.17	0.197	10.63
3♀ 4500 ppm HFC 143	66	253	1.16	6.9	1.43	56.5
	67	262	1.08	8.4	1.74	67.3
	68	243	1.16	7.2	1.64	71.0
	69	268	1.60	9.2	1.82	77.3
	70	255	1.11	8.5	1.70	77.0
	Mean	256	1.22	8.1	1.66	69.8
	SD	9.7	0.214	0.97	0.148	8.55

SD Standard deviation

APPENDIX 23

Rats dying or killed during the course of the study and killed at termination

Group:	1	2	3	4
Test substance:			HFC 143	
Level:	Control	2250 ppm	4500 ppm	9000 ppm

In this appendix, the macroscopic and microscopic findings relating to each animal are listed on one page. These findings are presented by an automated data collation system, and the following should be noted.

Particular care is taken during removal and processing of all protocol-scheduled tissues. Understandably, omissions or irregularities can occasionally occur, the most vulnerable tissues in this regard being parathyroid, thymus, male mammary gland and autolysed portions of the gastro-intestinal tract. For each animal, any tissues so affected are listed as not available for examination and a reason given for this.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 1♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Heart
Myocardial inflammation: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Liver; Kidneys; Epididymides; Testes; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 2♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart
Myocardial fibrosis: (Minimal , Focal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Kidneys; Epididymides; Testes. Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 3♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Heart; Kidneys; Epididymides; Testes; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 4♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Kidneys; Epididymides; Testes; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 5♂ (Terminal)

MACROSCOPIC FINDINGS

Pancreas
Congested

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Pancreas
Vascular congestion

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Kidneys; Epididymides, Testes; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 6♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: (Minimal) 7mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Epididymides; Testes

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 7♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Epididymides; Testes

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 8♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm
Lobular markings accentuated

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Epididymides; Testes

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 9♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Lymph Nodes - Deep Cervical
Enlarged: 3mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Epididymides; Testes

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 10♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Epididymides; Testes

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 11♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 12mm

Testes
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Intra-alveolar haemorrhage: (Area)

Epididymides
Spermatozoa absent from caput
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Moderate)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 12♂ (Terminal)

MACROSCOPIC FINDINGS

Kidneys
Irregular cortical scarring: (Minimal)

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Moderate)
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal) (Particularly at stages 14 to 3)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 13♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal) (Particularly at stages 14 to 3)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 14♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides

Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Moderate)
Degenerate round germ cells: (Minimal)

Testes

Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Trace)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal) (Particularly at stages 14 to 3)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 15♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Degenerate round germ cells: (Moderate)

Testes
Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal) (Particularly at stages 14 to 3)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 16♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm
Congested: (Minimal)

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 17♂ (Recovery)

MACROSCOPIC FINDINGS

Fur
Stained - periorbital region/s: (Right , Red , Brown)

Lymph Nodes - Cervical
Enlarged: 9mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Moderate)
Degenerate round germ cells: (Minimal)

Testes
Degenerate germ cells mainly spermatocytes: (Minimal)
Atrophic tubules lined only by Sertoli cells: (Marked)
Vacuoles in seminiferous epithelium: (Moderate)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 18♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Moderate)
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Moderate)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 19♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: (Minimal) 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 20♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: (Minimal) 7mm

Liver
Lobular markings accentuated

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Trace)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 21♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

Seminal Vesicles
Contents minimal: (Right horn)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Reduction/absence of tailed spermatids: (Moderate)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 22♂ (Terminal)

MACROSCOPIC FINDINGS

Liver
Necrotic lobe: Posterior caudate
Small lobe: Posterior caudate
Yellow lobe: Anterior caudate

Stomach Corpus Mucosa
Haemorrhagic depression: 1mm

Testes
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Moderate)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Marked)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Moderate)
Vacuoles in seminiferous epithelium: (Moderate)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 22♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 23♂ (Terminal)

MACROSCOPIC FINDINGS

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Moderate)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 24♂ (Terminal)

MACROSCOPIC FINDINGS

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Moderate)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Moderate)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 25♂ (Terminal)

MACROSCOPIC FINDINGS

Stomach Corpus Mucosa
Haemorrhagic depression: (Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Moderate)

Testes
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 26♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Trace)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 27♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: (Minimal) 6mm

Liver
Lobular markings accentuated

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Minimal)
Reduction/absence of round spermatids: (Minimal)
Reduction/absence of spermatocytes: (Minimal)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 28♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Marked)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Moderate)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 29♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 30♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Lungs
Adhesions: (Left) to ribs

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

Stomach Corpus Mucosa
Haemorrhagic depression: 2mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Moderate)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 30♂ - continued

MICROSCOPIC FINDINGS - continued

Testes

Atrophic tubules lined only by Sertoli cells: (Marked)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 31♂ (Terminal)

MACROSCOPIC FINDINGS

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Marked)
Reduction/absence of spermatogonia: (Moderate)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Multinucleate spermatocytes: (Trace)
Degenerate germ cells mainly spermatocytes: (Moderate)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Liver; Kidneys. Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 32♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Epididymides
Spermatozoa absent from caput
Reduced numbers of spermatozoa in cauda: (Marked)
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Moderate)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Minimal)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 32♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 33♂ (Terminal)

MACROSCOPIC FINDINGS

Stomach Antrum Mucosa
Haemorrhagic depression: 1mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Heart
Myocardial inflammation: (Minimal , Focal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Kidneys
Mineral casts in tubules at the corticomedullary junction: (Minimal)
Basophilic cortical tubules: (Minimal , Focal)

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 33♂ - continued

MICROSCOPIC FINDINGS - continued

Testes

Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Marked)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Multinucleate spermatocytes: (Trace)
Degenerate germ cells mainly spermatocytes: (Minimal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Adrenals; Stomach : (W.N.L.)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 34♂ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Thoracic Cavity
Contained serosanguineous fluid

Thymus
Oedematous

Lungs
Congested: (Right)

Liver
Lobular markings accentuated

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Five , Punctate)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Vascular congestion
Perivascular oedema
Intra-alveolar oedema

Thymus
Involution: (Moderate)
Vascular congestion

Liver
Centrilobular fibrosis: (Moderate)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 34♂ - continued

MICROSCOPIC FINDINGS - continued

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

Factors Contributory To Death
Vascular congestion and oedema in the lung

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 35♂ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Moderate)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 35♂ - continued

MICROSCOPIC FINDINGS - continued

Testes

Atrophic tubules lined only by Sertoli cells: (Moderate)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Moderate)
Reduction/absence of spermatogonia: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)
Multinucleate round spermatids: (Minimal)
Degenerate germ cells mainly spermatocytes: (Moderate)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Heart; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 36♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Marked)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Minimal)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 37♂ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Fur
Stained - perinasal region: Blood

Lungs
Haemorrhagic area: Left anterior lobe 5mm
Haemorrhagic: (Severe) Right anterior, posterior and azygos lobes

Stomach
Contents watery

Caecum
Contents dark

Adrenals
Congested

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Intra-alveolar haemorrhage: (Areas)

Caecum
Vascular congestion: (Minimal)

Factors Contributory To Death
Haemorrhage in the lung

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 37♂ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Adrenals : (W.N.L.); Stomach : (W.N.L.)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 38♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Atrophic tubules lined only by Sertoli cells: (Minimal)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Minimal)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 39♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Moderate)

Testes
Atrophic tubules lined only by Sertoli cells: (Marked)
Reduction/absence of tailed spermatids: (Marked)
Reduction/absence of round spermatids: (Moderate)
Reduction/absence of spermatocytes: (Minimal)
Vacuoles in seminiferous epithelium: (Moderate)
Multinucleate round spermatids: (Trace)
Degenerate germ cells mainly spermatocytes: (Trace)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 40♂ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

Testes
Small

Epididymides
Small

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Epididymides
Spermatozoa absent from caput
Spermatozoa absent from cauda
Degenerate round germ cells: (Minimal)

Testes
Degenerate germ cells mainly spermatocytes: (Minimal)
Atrophic tubules lined only by Sertoli cells: (Marked)
Vacuoles in seminiferous epithelium: (Moderate)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 41♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 11mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Kidneys
Basophilic cortical tubules: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Heart; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 42♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following observations were noted:

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 43♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Vascular congestion

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Bile duct hyperplasia: (Trace)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Heart; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 44♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

- Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Liver; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 45♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonitis: (Minimal , Focal)

Lymph Nodes - Cervical
Lymphoid proliferation: (Moderate)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Heart; Liver; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 46♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 47♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 48♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 49♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: Air Control

Rat No/Sex: 50♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Uterus
Fluid distension: (Minimal)

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 51♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 52♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 10mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 53♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 54♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250.ppm

Rat No/Sex: 55♀ (Terminal)

MACROSCOPIC FINDINGS

Incisors
Pale: (Lower)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 56♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 57♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 58♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 59♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 2250 ppm

Rat No/Sex: 60♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: (Minimal) 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 2mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 61♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 62♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 9mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 63♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Tracheobronchial
Not visible

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 64♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 65♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Lungs

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 66♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 67♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 68♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

Liver
Pale subcapsular area/s - median cleft: (One) 1mm

Uterus
Fluid distension

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 69♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 8mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 4500 ppm

Rat No/Sex: 70♀ (Recovery)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged: 7mm

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 71♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities were seen in the animal

MICROSCOPIC FINDINGS

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Liver; Kidneys; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 72♀ (Terminal)

MACROSCOPIC FINDINGS

Thymus
Congested: (Minimal)

Liver
Adhesions: Left lobe to median lobe

Kidneys
Pale cortical area/s: (Right , One) 2mm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Thymus
Vascular congestion

Liver
Adhesion

Kidneys
Cortical scarring: (Minimal , Focal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 73♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

Spleen
A pale capsular area: 3mm

Kidneys
Irregular cortical scarring: (Minimal)
Adhesions: Left to pancreas and right to right posterior lobe of liver

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Spleen
Capsular thickening: (Minimal)

Kidneys
Pyelitis: (Minimal , Bilateral)
Cortical scarring: (Minimal , Focal)
Mineral casts in tubules at the corticomedullary junction: (Minimal)
Urothelial hyperplasia in the pelvis: (Minimal , Bilateral)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 73♀ - continued

MICROSCOPIC FINDINGS.- continued

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 74♀ (Terminal)

MACROSCOPIC FINDINGS

Lymph Nodes - Cervical
Enlarged

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lymph Nodes - Cervical
Lymphoid proliferation: (Minimal)

Liver
Extramedullary haemopoiesis: (Minimal , Focal)

Kidneys
Mineral casts in tubules at the corticomedullary junction: (Moderate)
Cortical collecting ducts lined by clear cells: (Minimal)

The following tissues were considered normal:

Nasal Turbinates; Larynx; Trachea; Tracheal Bifrucation; Lungs; Heart; Adrenals

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm .

Rat No/Sex: 75♀ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Fur
Stained - perinasal region: (Minimal , Brown)
Moist - perinasal region

Lungs
Not collapsed
Firm
Congested: (Severe)

Stomach
Contents watery

Stomach Antrum Mucosa
White nodules, near to limiting ridge: (Two) 1mm

Adrenals
Congested: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Vascular congestion
Perivascular oedema

Stomach
Focus of ectopic non-glandular epithelium within the glandular mucosa

Factors Contributory To Death
Vascular congestion and oedema in the lung

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 75♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Adrenals : (W.N.L.)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 76♀ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Lungs
Congested: (Minimal)

Small Intestine
Distended: (Minimal)

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Increased numbers of alveolar macrophages: (Minimal)
Vascular congestion
Perivascular oedema
Intra-alveolar haemorrhage: (Areas)

Factors Contributory To Death
Vascular congestion and oedema in the lung

The following tissues were considered normal:

Duodenum : (W.N.L.); Jejunum : (W.N.L.)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 77♀ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Brain
Congested

Lungs
Not collapsed

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Vascular congestion
Perivascular oedema

Brain
Vascular congestion

Factors Contributory To Death
Vascular congestion and oedema in the lung

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 78♀ (Intercurrent)

MACROSCOPIC FINDINGS

Died awaiting post mortem

Thoracic Cavity
Contained serous fluid: Clear

Thymus
Oedematous

Lungs
Congested
Oedematous

Liver
Pitted
Lobular markings accentuated

Caecum
Contents firm

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Pneumonia: (Moderate)
Vascular congestion
Perivascular oedema

Thymus
Involution: (Moderate)

Liver
Centrilobular hepatocyte necrosis: (Minimal)

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 78♀ - continued

MICROSCOPIC FINDINGS - continued

Factors Contributory To Death
Vascular congestion and oedema in the lung

The following tissues were considered normal:

Caecum : (W.N.L.)

Pathologist: J.M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 79♀ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Thoracic Cavity
Contained serous fluid: Clear

Lungs
Congested: (Severe)

Liver
Lobular markings accentuated

Adrenals
Congested

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Vascular congestion
Perivascular oedema
Intra-alveolar haemorrhage: (Areas)

Liver
Centrilobular hepatocyte necrosis: (Minimal)

Factors Contributory To Death
Haemorrhage in the lung

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 79♀ - continued

MICROSCOPIC FINDINGS - continued

The following tissues were considered normal:

Adrenals : (W.N.L.)

Pathologist: J M.Offer

APPENDIX 23

(Pathology - continued)

Compound: HFC 143

Dosage Level: 9000 ppm

Rat No/Sex: 80♀ (Intercurrent)

MACROSCOPIC FINDINGS

Found dead

Lymph Nodes - Inguinal
Congested: (Right)

Thoracic Cavity
Contained serosanguineous fluid

Thymus
Oedematous

Lungs
Congested: (Severe)
Oedematous

Lymph Nodes - Tracheobronchial
Not visible

Liver
Lobular markings accentuated

Stomach
Empty

Stomach Corpus Mucosa
Depressions: (A few , Punctate) Haemorrhagic

Adrenals
Congested

All the other organs and tissues appeared normal.

APPENDIX 23

(Pathology - continued)

Rat No/Sex: 80♀ - continued

MICROSCOPIC FINDINGS

The following observations were noted:

Lungs
Increased numbers of alveolar macrophages: (Minimal)
Perivascular oedema
Intra-alveolar haemorrhage: (Areas)
Intra-alveolar oedema: (Areas)

Thymus
Involution: (Moderate)

Liver
Centrilobular hepatocyte necrosis: (Minimal)

Adrenals
Sinusoidal congestion

Stomach
Erosion of glandular epithelium: (Multiple)

Factors Contributory To Death
Haemorrhage in the lung

Tissues not available for examination were:

Lymph Nodes - Inguinal : (Not seen)

Pathologist: J.M.Offer

APPENDIX 24

Inorganic fluoride - contract laboratory protocol, methodology and results

SPONSOR

Huntingdon Research Centre Limited

(HRC)

STUDY SCHEDULE

HRC Schedule PDR585

STUDY OBJECTIVE

Analysis of Fluoride Concentration
in Rat Urine

TESTING FACILITY

BUTTERWORTH LABORATORIES LIMITED

STUDY SCHEDULE NUMBER

BL 1/1019 (94)

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

STATEMENT OF DATA CONFIDENTIALITY

All data generated by Butterworth Laboratories Limited will be held in our archives for a period of not less than SIX years.

As with all work undertaken by Butterworth Laboratories Limited, customer confidentiality will be observed.

STUDY DIRECTOR'S AUTHENTICATION

I, the undersigned, accept responsibility for the conduct of the study and confirm that analyses were undertaken in compliance with the Principles of Good Laboratory Practice.

Signed.....

David Riches BSc C Chem MRSC
Study Director

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (93)

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

PERSONNEL INVOLVED

D.J. Hawkins BSc C.Biol MIBiol
Analytical Chemist

D.A. Riches BSc C.Chem MRSC
Senior Manager - Environmental
Study Director

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

INTRODUCTION

The objective of this study was to determine the Fluoride concentration in samples of Rat Urine.

It was proposed to analyse the urine using a Fluoride ion selective electrode based on our In-house method BLM 143 "The Determination of Fluoride by Ion Selective Electrode in Aqueous Solutions" and the published method stated in the Protocol. Prior to the commencement of the Study a validation exercise was performed to confirm the suitability of this method for urine.

The validation exercise on a control urine sample produced the following conclusions:

1. The electrode shows repeatable calibration in the range 0.05 mg/l to 2.0 mg/l on different days and with different operators.

2. The electrode response is repeatable for different sample solutions to ± 1 mV.

3. The mean result on the control rat urine was 1.65 mg/L ± 0.1 mg/l (95% confidence). The estimated precision over the whole calibration range was $\pm 6\%$.

4. The limit of detection of Fluoride in urine is 0.25 mg/l.

As a result of this validation a new In-house method BLM 147 was written specifically for the analysis of Fluoride in Rat Urine, and accepted by Huntingdon Research Centre Limited.

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

EXPERIMENTAL PROCEDURE

Equipment/Instrumentation

Kent Industrial Instruments 8001-2 Fluoride Ion Selective Electrode

BDH Double Junction Glass Reference Electrode

UltroLab 2100 pH Meter

Magnetic Stirrer and stirring fleas

Plastic beakers 25-40ml capacity

Mettler AT261 5 - figure analytical balance

Grade A Volumetric flasks (100ml and 1000ml)

Grade A Pipettes (2ml, 5ml, 10ml, and 25ml)

Grade A 50ml Burette

Reagents and Standards

AnalaR grade Sodium Fluoride

Total Ionic Strength Adjustment Buffer (TISAB) - pre prepared and supplied by BDH (Product No. 16084)

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Analytical Procedure

Calibration and analysis was carried out in accordance with BLM 147 "The Determination of Fluoride by Ion Selective Electrode in Rat Urine".

Where possible the samples were prepared by diluting 2ml of urine with 8ml of TISAB (ie a 1 in 5 dilution). As a minimum of 10 ml of sample solution was required for analysis, where necessary, greater dilutions were made due to low sample volumes being available.

CONCLUSION

The Fluoride concentrations reported, were found to be in the range of 1 - 7 mg/L. The analysis of duplicates show good reproducibility.

Varying amounts of deposit were noticed in all the samples. The results obtained refer to soluble Fluoride only.

APPENDIX 24

(Inorganic fluoride - continued)

Butterworth Laboratories Ltd
Analytical & Consulting Chemists

54-56 Waldegrave Road,
Teddington, Middlesex
TW11 8LG UK

Telephone: 081-977 0750
Fax: 081-943 2624

Certificate of Analysis

Study Schedule No. BL 1/1019 (94)

Our Ref:DB/jhs 23 March 1994

Huntingdon Research Centre Limited
P O Box 2
Huntingdon
Cambridgeshire
PE18 6ES

For the attention of Mr D.M. Coombs

Samples of: Rat Urine

BLL Study Schedule No: BL 1/1019 (94)

HRC Reference: PDR585 - Inorganic Fluoride Analysis of Rat Urine

Date Received: 2nd February 1994

Results

For samples dated by HRC as 14th January 1994.

BLL Reference	HRC Reference Group 1 Male	Fluoride expressed as F Results in mg/L
BL 2/0070 (94)	1	1.8
BL 2/0071 (94)	2	1.5
BL 2/0072 (94)	3	1.6
BL 2/0073 (94)	4	1.6
BL 2/0074 (94)	5	3.2
BL 2/0075 (94)	6	1.8
BL 2/0076 (94)	7	1.2, 1.1
BL 2/0077 (94)	8	1.3
BL 2/0078 (94)	9	1.6
BL 2/0079 (94)	10	1.3

NAMAS TESTING No 0215

GLP Compliant Laboratory

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 2 Male	Fluoride expressed as F Results in mg/L
BL 2/0080 (94)	11	3.8
BL 2/0081 (94)	12	3.5
BL 2/0082 (94)	13	4.4
BL 2/0083 (94)	14	2.4
BL 2/0084 (94)	15	4.2
BL 2/0085 (94)	16	3.1
BL 2/0086 (94)	17	3.2
BL 2/0087 (94)	18	2.4
BL 2/0088 (94)	19	3.5
BL 2/0089 (94)	20	3.3
BLL Reference	**HRC Reference Group 3 Male**	**Fluoride expressed as F Results in mg/L**
BL 2/0090 (94)	21	5.0
BL 2/0091 (94)	22	4.2
BL 2/0092 (94)	23	4.0
BL 2/0093 (94)	24	4.5
BL 2/0094 (94)	25	4.0
BL 2/0095 (94)	26	4.0
BL 2/0096 (94)	27	5.3
BL 2/0097 (94)	28	3.3
BL 2/0098 (94)	29	3.7
BL 2/0099 (94)	30	3.7, 3.8

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 4 Male	Fluoride expressed as F Results in mg/L
BL 2/0100 (94)	31	4.2
BL 2/0101 (94)	32	3.6
BL 2/0102 (94)	33	3.7
BL 2/0103 (94)	35	3.6
BL 2/0104 (94)	36	3.1, 2.7, 2.8
BL 2/0105 (94)	38	2.3
BL 2/0106 (94)	39	5.1
BL 2/0107 (94)	40	3.7

BLL Reference	HRC Reference Group 1 Female	Fluoride expressed as F Results in mg/L
BL 2/0108 (94)	41	2.3
BL 2/0109 (94)	42	2.2
BL 2/0110 (94)	43	1.7
BL 2/0111 (94)	44	1.4
BL 2/0112 (94)	45	1.7
BL 2/0113 (94)	46	1.5
BL 2/0114 (94)	47	1.6
BL 2/0115 (94)	48	1.4
BL 2/0116 (94)	49	2.0
BL 2/0117 (94)	50	1.2

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 2 Female	Fluoride expressed as F Results in mg/L
BL 2/0118 (94)	51	2.8
BL 2/0119 (94)	52	2.1
BL 2/0120 (94)	53	2.9
BL 2/0121 (94)	54	2.7
BL 2/0122 (94)	55	2.6
BL 2/0123 (94)	56	4.2
BL 2/0124 (94)	57	2.8
BL 2/0125 (94)	58	2.1
BL 2/0126 (94)	59	2.6
BL 2/0127 (94)	60	2.1
BLL Reference	**HRC Reference Group 3 Female**	**Fluoride expressed as F Results in mg/L**
BL 2/0128 (94)	61	3.2
BL 2/0129 (94)	62	4.0
BL 2/0130 (94)	63	4.5
BL 2/0131 (94)	64	2.7
BL 2/0132 (94)	65	1.8, 1.8
BL 2/0133 (94)	66	3.0
BL 2/0134 (94)	67	3.3
BL 2/0135 (94)	68	3.1
BL 2/0136 (94)	69	4.4
BL 2/0137 (94)	70	4.9

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 4 Female	Fluoride expressed as F Results in mg/L
BL 2/0138 (94)	71	7.0
BL 2/0139 (94)	72	3.1
BL 2/0140 (94)	73	3.4
BL 2/0141 (94)	74	6.0

Samples analysed in accordance with In-house method BLM 147.

DAVID A. RICHES
Study Director
for Butterworth Laboratories Limited

APPENDIX 24

(Inorganic fluoride - continued)

Butterworth Laboratories Ltd
Analytical & Consulting Chemists

54-56 Waldegrave Road,
Teddington, Middlesex
TW11 8LG UK

Telephone: 081-977 0750
Fax: 081-943 2624

Certificate of Analysis

Study Schedule No. BL 1/1019 (94)

Our Ref:DB/jhs 23 March 1994

Huntingdon Research Centre Limited
P O Box 2
Huntingdon
Cambridgeshire
PE18 6ES

For the attention of Mr D.M. Coombs

Samples of: Rat Urine

BLL Study Schedule No: BL 1/1019 (94)

HRC Reference: PDR585 - Inorganic Fluoride Analysis of Rat Urine

Date Received: 2nd February 1994

Results

For samples dated by HRC as 28th January 1994.

BLL Reference	HRC Reference Group 1 Male	Fluoride expressed as F Results in mg/L
BL 2/0143 (94)	6	1.7
BL 2/0144 (94)	7	1.1
BL 2/0145 (94)	8	1.8, 1.7
BL 2/0146 (94)	9	1.6
BL 2/0147 (94)	10	1.1

BLL Reference	HRC Reference Group 2 Male	Fluoride expressed as F Results in mg/L
BL 2/0148 (94)	16	1.6
BL 2/0149 (94)	17	1.6
BL 2/0150 (94)	18	1.4
BL 2/0151 (94)	19	1.6
BL 2/0152 (94)	20	1.7

GLP Compliant Laboratory

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 3 Male	Fluoride expressed as F Results in mg/L
BL 2/0153 (94)	26	1.5
BL 2/0154 (94)	27	3.4
BL 2/0155 (94)	28	1.7
BL 2/0156 (94)	29	2.6
BL 2/0157 (94)	30	2.0
BLL Reference	**HRC Reference Group 4 Male**	**Fluoride expressed as F Results in mg/L**
BL 2/0158 (94)	36	2.0
BL 2/0159 (94)	38	1.7
BL 2/0160 (94)	39	1.7
BL 2/0161 (94)	40	3.6
BLL Reference	**HRC Reference Group 1 Female**	**Fluoride expressed as F Results in mg/L**
BL 2/0162 (94)	46	1.1
BL 2/0163 (94)	47	1.1
BL 2/0164 (94)	48	1.4
BL 2/0165 (94)	49	1.4
BL 2/0166 (94)	50	1.1, 1.2
BLL Reference	**HRC Reference Group 2 Female**	**Fluoride expressed as F Results in mg/L**
BL 2/0167 (94)	56	1.4
BL 2/0168 (94)	57	1.4
BL 2/0169 (94)	58	1.5
BL 2/0170 (94)	59	1.7
BL 2/0171 (94)	60	1.4

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BLL Reference	HRC Reference Group 3 Female	Fluoride expressed as F Results in mg/L
BL 2/0172 (94)	66	2.1
BL 2/0173 (94)	67	1.6, 1.6
BL 2/0174 (94)	68	2.0
BL 2/0175 (94)	69	2.4
BL 2/0176 (94)	70	1.7

Samples analysed in accordance with In-house method BLM 147.

DAVID A. RICHES
Study Director
for Butterworth Laboratories Limited

APPENDIX 24

(Inorganic fluoride - continued)

PROTOCOL

SCHEDULE NO:

BL 1/1019 (94)

SPONSOR

Huntingdon Research Centre Limited
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES

TEST MATERIAL

Rat Urine

OBJECTIVE OF STUDY

Inorganic Fluoride Analysis.

TESTING FACILITY

Butterworth Laboratories Limited
54-56 Waldegrave Road, Teddington, Middlesex TW11 8LG

APPENDIX 24

(Inorganic fluoride - continued)

STARTING DATE

Within 72 hours of acceptance of Method Validation data.

SAMPLE STORAGE

Samples will be stored at -20°C until required for analysis. On completion of the analysis, samples will be stored at -20°C until Analytical Data has been accepted by the Sponsor.

COMPLETION DATE

A copy of the Certificate of Analysis will be issued as an interim report, with the original Certificate of Analysis being bound in the final GLP report to be issued within 25 working days of completion of the analytical work.

METHODS TO BE USED

In-house Method to be developed, validated and agreed with Sponsor in advance of the Study being started.

Method to be based on Ion Selective Electrode method published in J.Lab & Clin. Med. August 1969, by L. Singer, W.D. Armstrong and J.J. Vogel. Determination of fluoride content of urine by electrode potential measurements.

APPENDIX 24

(Inorganic fluoride - continued)

DISPOSAL OF SAMPLES

Any sample material not used for analysis will be returned to the Sponsor for disposal, unless written instructions are provided for the safe disposal of the material by Butterworth Laboratories Limited.

RECORDS TO BE ARCHIVED

Details of: Sample/batch reference numbers. Instrumentation used; standards prepared; copies of all raw data generated. The current archiving period is six years, and all archives to be kept on the premises of Butterworth Laboratories Limited.

The Final Report issued to the Sponsor will contain all study specific raw data, together with authenticated photocopies of all applicable supporting data and In-house methodology.

STUDY DIRECTOR

David Riches C.Chem MRSC
Senior Manager

MONITORING SCIENTIST

Derek Coombes
Huntingdon Research Centre Limited

APPENDIX 24

(Inorganic fluoride - continued)

QUALITY ASSURANCE

The Quality Assurance Department of Butterworth Laboratories Limited will audit all analytical results and undertake periodic monitoring of this Protocol. The dates of the audits and aspects monitored will be contained in the Final Report.

Documentation for this Protocol will be open at all times to inspection by the Sponsor.

ACCREDITATION

The study will be conducted in compliance with the Principles of Good Laboratory Practice (GLP) as set forth in 'Good Laboratory Practice, the United Kingdom Compliance Programme, Department of Health and Social Security, 1986, and subsequent revision, Department of Health, 1989'.

Butterworth Laboratories Limited hold current NAMAS accreditation and all aspects of this study will also be carried out in accordance with this Quality Standard.

Accepted on behalf of Butterworth Laboratories Limited

Signed..... Date..1/2/94.
David Riches C.Chem MRSC
Senior Manager
Study Director

Accepted on behalf of Huntingdon Research Centre Limited

Signed..... Date.01/02/94
Derek Coombes
Monitoring Scientist

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

METHOD NO: BLM 147

ISSUE NO: 1

DATE ISSUED: 25 February 1994

ORIGINATOR: D Hawkins

AUTHORISED: D E Butterworth

TITLE: **The Determination of Fluoride by Ion Selective Electrode in Rat Urine.**

INTRODUCTION

This ion selective electrode method has been developed to determine the concentration of Fluoride in Rat Urine at concentratons between 0.05 mg/l and 100 mg/l in the test solutions. Samples are diluted in a Total Ionic Strengths Adjustment Buffer to release free Fluoride ions and to provide a suitable pH for analysis. In theory, there is no upper limit to the method's range as samples may be diluted many times before analysis.

The fluoride electrode is a chemical sensor in which the detector is a doped single crystal of lanthanum fluoride across which a potential is developed in the presence of fluoride ions. It is normally used with a standard calomel reference electrode and a high impedence millivolt meter or pH meter.

The electrode responds to activity rather than the concentration of the fluoride ions and to ensure a constant relationship between activity and concentration, samples and calibration standards must be adjusted to a constant ionic strength. They must also be buffered at a suitable pH value to prevent interference by hydroxide ions and also the formation of unionised HF under acid conditions. The buffer reagent contains metal-complexing agents (de-complexing agents) to release free fluoride ion from certain metal-fluoride complexes, to which the electrode does not respond. Simple fluoro-silicates are rapidly hydrolysed in water and are detected as fluoride by the electrode.

LITERATURE REFERENCES

Standing Committee of Analysts "Fluoride in Waters, Effluents, Sludges, Plants and Soils 1982" (HMSO)

Kent Industrial Measurements instruction manual for Model 8001-2 Fluoride Electrode

Russell PH Limited "The Guide to Ion Analysis"

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

METHOD NO: BLM 147 **ISSUE NO: 1**

METHOD VALIDATION

The method which is essentially that as published in the Standing Committee of Analysts "Blue Book" method has been assumed to have been validated. When either the Fluoride or Calomel electrode has to be replaced, due to breakage or deterioration of performance, the performance of the new electrode system is thoroughly checked by the determination of standards produced in DI water and checked by the alternative technique of Ion Chromatography.

Fluoride Ion Selective Electrode Reference No: LP1 and BDH Calomel Electrode No: 309/1030/04 System was introduced on 1 February 1994.

Validation data for electrode system archived as part of HRC GLP Study: BL 1/1019 (94)
Standards are measured before each run and the instrument calibrated.

EQUIPMENT

Kent Industrial Instruments 8001-2 Fluoride Ion Selective Electrode (or equivalent)

Double or Single Junction (silver/silver chloride or calomel) reference electrode

Expanded scale pH meter with a mV reading capacity

Magnetic stirrer and stirring fleas

Plastic beakers (50-60 ml)

5-Figure Analytical Balance

Grade A Volumetric Flasks (100ml and 1000ml)

Grade A Pipettes (2ml, 5ml, 10ml and 25ml)

Grade A 50ml Burette

REAGENTS

Primary Stock Fluoride Standard Solution (1 ml = 1000 μg F (1000 mg/l F))

Dry Sodium Fluoride for four hours at 105°C and cool in a desiccator. Weigh out accurately 2.210 g ($\pm$0.001 g) and dissolve in water. Transfer to a one litre volumetric flask and make up to the mark with water. Transfer immediately to a polythene bottle, this solution can be stored for no longer than three months.

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

CONTROLLED DOCUMENT

METHOD NO: BLM 147 **ISSUE NO: 1**

Secondary Stock Fluoride Standard Solution (1 ml = 100 µg F^- (100 mg/l F^-))

Take 10 ml of the primary standard and pipette into a 100 ml volumetric and make up to the mark with water and transfer immediately to a new 200ml sterile plastic pot. Prepare this standard freshly each day.

Working Standards

Each standard is made up to 100 ml in a volumetric flask using TISAB, transferred to a new sterile plastic pot and should be prepared freshly each day.

Starting Standard	Volume Taken	Make up to with TISAB	Concentration of Working Standard
Primary Standard 1000 mg/l F^-	10 ml	100 ml	100 mg/l F^-
Secondary Standard 100 mg/l F^-	10 ml	100 ml	10 mg/l F^-
Working Standard 10 mg/l F^-	10 ml	100 ml	1 mg/l F^-
Working Standard 1 mg/l F^-	10 ml	100 ml	0.1 mg/l F^-

Total Ionic Strength Adjustment Buffer (TISAB)

Pre-prepared and supplied by BDH (Prod 16084).

REFERENCE MATERIALS

AnalaR grade or equivalent: Sodium Fluoride

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

METHOD NO: BLM 147 **ISSUE NO: 1**

PERFORMANCE CHARACTERISTICS

Precision	± 6% test solution concentration
Accuracy	Accuracy varies as the electrode response is logarithmic; the electrode can measure a response ± 1mV
Analytical Range	0.05 mg/l F^- to 100 mg/l F- in test solution
Linear Range	Response vs log concentration is linear between 0.1 and 100 mg/l F- in test solution
Determination Limit	0.05 mg/l F^- in test solution (0.25 mg/l F^- in Urine Sample)
Sensitivity	A change in 1 mV = 0.01 mg/l at low concentrations.
Limit of Detection	0.05mg/l

INTERFERENCES

Hydroxyl ions which should be buffered against by using TISAB.

Chemical species that reduce the concentrations of Fluoride ions in the sample, eg Polyvalent cations such as Fe, Ca, Al. The CDTA in the TISAB should decomplex combinations of Fluoride and these species.

Borates: if Borates are known to be present, separation by diffusion or distillation is required.

SAMPLE PREPARATION

Samples should be stored in plastic or polythene containers and should be analysed as soon as possible. Provided the sample pH is between 5 and 6, the proportion of TISAB:sample should be 5:1. With only small sample volumes of Rat Urine available, it is recommended that 2ml of Sample be diluted to 10ml TISAB.

ANALYTICAL PROCEDURE

1 Ion selective electrodes are sensitive instruments and various instability effects may be seen. Refer to the Standing Committee of Analysts "Fluoride in Waters, Effluents, Sludges, Plants and Soils 1982", section A8, page 14, before carrying out the experimental procedure.

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

CONTROLLED DOCUMENT

METHOD NO: BLM 147 **ISSUE NO: 1**

ANALYTICAL PROCEDURE Cont.

2 A constant temperature should be maintained throughout the run.

(a) Connect the ion selective electrode and the reference electrode to the pH meter and stand in TISAB overnight

(b) Switch on the pH meter and note the reading in mV. This is the background reading which must be achieved before a determination is made

(c) Prepare the working standards as detailed above

(d) Using a Grade A pipette add 2ml of sample to a plastic beaker and add 8ml of TISAB using a burette and allow to stand for 15 minutes (check the pH - see note (j))

(e) Pour ≈ 10 ml of each working standard into plastic beakers and add a PTFE magnetic stirring flea to each beaker (sample and standards). (See note (l))

(f) Place the beaker onto the magnetic stirrer and establish a constant stirring rate so that no bubbles or vortex are formed. Fix the speed control on the stirrer to prevent the stirring rate from changing throughout the run

(g) Insert the electrode into the test solution, taking care not to trap any bubbles under the electrodes

(h) Leave for four minutes and switch off the magnetic stirrer (at the on/off switch). After one minute note the mV reading to nearest 1 mV. After a further minute note the reading again and if the readings are the same, record them. If not, repeat the process

(i) Lift the electrodes out of the solutions and rinse thoroughly with deionised water. Dry with a clean tissue and immerse into a beaker containing TISAB. Allow the mV reading to reach the background level, rinse, dry and repeat from stage (f) onwards

(j) Analyse the samples in the same fashion as for the standards. The pH should be checked using pH strips. A pH in the range of 5-6 is desired. If this range is not achieved, a greater proportion of TISAB to sample may be used.

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

BUTTERWORTH LABORATORIES LIMITED - IN-HOUSE METHOD

METHOD NO: BLM 147 **ISSUE NO: 1**

ANALYTICAL PROCEDURE Cont.

2 (k) A calibration graph is plotted:

y axis (on a log scale) = concentration in the test solution

x axis (on a linear scale) = - mV reading

From this graph (or from a statistical analysis carried out on a computer, based on an equation formed from the calibration data) the sample test solution concentrations can be calculated

(l) If the lower limit of detection of 0.05 mg/l F^- in the test solution is required, 5ml of the 0.1 mg/l F- working standard is pipetted into a beaker with 5ml of TISAB to prepare the relevant standard

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

2/3/94 DM. 319

File: 020394

xy math correlation co = 0.9999999904

Sample / std.	volume taken mls	volume made up to with TISAB.	mV 1min	mV 2min	Conc of F⁻ in test solution mg/L.	PH. in test solution	Concn in Sample
10mg/L F⁻	5mL	10mL			5.0mg/L	5.5	~~5.5~~
10mg/L F⁻	2mL	10mL	132	-132	2.0mg/L.		
1.0mg/L F⁻	5mL	10mL	.97	-97	0.5mg/L		
1.0mg/L F⁻	2mL	10mL	.76	-76	0.2 mg/L.		
0.1mg/L F	5mL	10mL	49	-49	0.05mg/L.		
210070	2mL	10mL	89	-89	0.356	5.5	1.78
210071			.85	-85	0.300		~~0~~1.5
210072			-86	-86	0.313		1.57
210073			86	-86	0.313		1.57
210074			-103	-103	~~0.723~~ 0.640 DM (typing error)		3.20
210075			-89	-89	0.356		1.78
210076			-80	-80	0.240		1.20
210077			-82	-82	0.262		1.31
210078			-87	-87	0.327		1.64
210079			-81	-81	0.251		1.26
210080			107	107	0.752		3.76
210081			-105	-105	0.694		3.47

checked DQ 12/3/94

318 24/3/94

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample ident	volume used mls	volume made up to mLs	mV 1 min	mV 2 min	Concn of F⁻ in test solution mg/L F⁻	PH	Concn of F⁻ in Sample mg/L F⁻
1.0mg/L F	5mL	10mL	-98	-98	0.5mg/L	5.5	
2/0082	2mL		-111	-111	0.883		4.42
2/0083			-96	-96	0.479		2.40
2/0084			-110	-110	0.848		4.24
2/0085			-102	-102	0.614		3.07
2/0086			-103	-103	0.640		3.20
2/0087			-96	-96	0.479		2.40
2/0088			-105	-105	0.694		3.47
2/0089			-104	-104	0.666		3.33
2/0090			-114	-114	0.994		~~4.47~~ 4.97
2/0091			-110	-110	0.848		4.24
1.0mg/L F	5mL	10mL	-98	-98			

Checked [initials] 18/3/94

[initials] 24/3/94

Study Record Book

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

3/3/94 DM. Temp = 25°C

Sample Std	Volume add ml	Volume made up to with TISAB	mV 1 min	mV 2 min	mg of F in Std in mg/L	PH	Conc of F in Sample mg/L
10 mg/L F	2 mL	10mL	-136	-136	2.0 mg/L	5.5	
1.0 mg/L F	5mL	10mL	-96	-94	0.5 mg/L		
1.0 mg/L F	2mL	10mL	-80	-80	0.2 mg/L		
0.1 mg/L F	5mL	10mL	-39	-39	0.05 mg/L		
	NB	Remake Stds. as	Calibration	is poor			
10 mg/L F	2mL	10mL	-138	-138	2.0 mg/L	5.5	
1.0 mg/L F	5mL		-102	-103	0.5 mg/L		
1.0 mg/L F	2mL		-81	-81	0.2 mg/L		
0.1 mg/L F	5mL		-54	-54	0.05 mg/L		
210092	2mL	10mL	-115	-115	0.805	5.5	4.025
210093			-118	-118	0.906	5.5	4.53
210094			-115	-115	0.805	5.5	4.025
210095			-115	-115	0.805		4.025
210096			-122	-122	1.061		5.305
210097			-110	-110	0.661		3.305

Checked QL 8/3/94

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Study record

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample Id	Volume mls	Volume made (upto in 250ml)	mV 1 min	mV 2 min	pH	Conc of F⁻ in test Solution mg/l F⁻	Conc of F⁻ in Sample
2/0001	2ml	10ml	-113	-113	5.5	0.744	3.72
2/0002			-113	-113	5.5	0.744	3.72
2/0100			-116	-111		0.937	4.185
2/0101			-112	-112		0.715	3.575
2/0102			-113	-113		0.744	3.7
2/0103			-112	-112		0.715	3.58
2/0104			-108	-108		0.610	3.05
2/0105			-101	-101		0.461	2.31
2/0106			-121	-121		1.020	5.10
2/0107			-113	-113		0.744	3.72
2/0108			-101	-101		0.461	2.31 24/3/94
2/0109	1ml		-83	-83		0.218	2.18
2/0110	2ml		-93	-93		0.333	1.67
2/0111		10.5ml	-89	-89		0.282	1.41
2/0112			-92	-92		0.320	1.68

Checked 18/3/94

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24/3/94

Study Record Book

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

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DSM 8/3/94 Temp = 24°C

Sample / Standard	Volume used mL	Volume made up to 15 mL with	mV reading 1 min	mV reading 2 min	pH of Solution	Conc of F⁻ in test Solution mg/L F⁻	Conc in Sample mg/L F⁻
10.0 mg/L F⁻	2 mL	10 mL	-126	-126	5.5	2 mg/L F⁻ (Rpt)	Too low
1.0 mg/L F⁻	5 mL	10 mL	-102	-102		0.5 mg/L F⁻	1.45
1.0 mg/L F⁻	2 mL	10 mL	-79	-79		0.2 mg/L F⁻	1.57
0.1 mg/L F⁻	5 mL	10 mL	-51	-51		0.05 mg/L F⁻	1.39
10.0 mg/L F⁻	2 mL	10 mL	-137	-137	5.5	2 mg/L F⁻	1.98
2/0113	2 mL	10 mL	-88	-88	5.5	0.289	1.23
2/0114			-90	-90		0.313	2.78
2/0115			-87	-87		0.277	2.14
2/0116			-96	-96		0.396	2.93
2/0117			-84	-84		0.246	2.71
2/0118		13	-99	-99		0.428	2.61
2/0119		10	-98	-98		0.428	4.16
2/0120			-106	-106		0.585	2.82
2/0121			-104	-104		0.541	2.06
2/0122			-103	-103		0.521	2.61
2/0123			-115	-115		0.831	
2/0124			-105	-105		0.563	
2/0125	2mL	10	-98	-97	6.5-5	0.412	
2/0126			-103	-103	5.5	0.521	

Cloudy 18/3/94 DE

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Study Record Sheet

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample / Standard	volume used ml	volume made up to in TISAB	MV Reading 1 min	MV Reading 2 min	pH	Concn in mg/L F⁻ of test Solution	Concn in 3:1 Sample mg/L F⁻
2/0127	2 mL	10	-98	-98	5.5	0.428	2.14
2/0128			-108	-108		0.632	3.16
2/0129			-114	-114		0.799	4.0
2/0130			-117	-117		0.898	4.49
2/0131			-104	-104		0.541	2.71
2/0132			-94	-94		0.366	1.83
2/0133			-107	-107		0.608	3.04
2/0134			-109	-109		0.657	3.29
To check reproducibility within a run.							
1.0 mg/L F⁻	5 mL	10mL	-100	-100	5.5	0.463	0.93 = 93%

24/3/94

Charles QC 28/3/94

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Study Record Book

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

DMM 9/3/94 Temperature = 23°C

Sample / Standard	Volume used mL	Volume made up to TISAB mL	mV reading 1 min	mV reading 2 min	pH	Conc in test soln mg/L F⁻	Conc in Sample mg/L F⁻
10.0 mg/L F	2 mL	10 mL	-138	-139	5.5	2 mg/L	
1.0 mg/L F	5 mL		-103	-103		0.5 mg/L	
1.0 mg/L F	2 mL		-78	-78		0.2 mg/L	
0.1 mg/L F	5 mL		-51	-51		0.05 mg/L	
2/0135	2 mL		-109	-109		0.624	3.12
2/0136			-118	-118		0.879	4.40
2/0137	1 mL		-121	-121		0.988	4.94
2/0138			-112	-112		0.698	6.98
2/0139	2 mL		-109	-109		0.624	3.12
2/0140			-111	-111		0.672	3.36
2/0141	0.4 mL		-105	-105		0.538	5.98
2/0142	2 mL					see validation sample.	
2/0143			-92	-92		0.336	1.68
2/0144			-81	-81		0.224	1.12
2/0145			-94	-94		0.360	1.80
2/0146			-90	-90		0.312	1.56
2/0147	2 mL	10 mL	-81	-81	5.5	0.224	1.12

checked QR 8/3/94

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample/ Standard	volume taken	volume made up to 10mL	mV reading 1 min	mV reading 2 min	pH	Conc in test Solution mg/L F⁻	Conc in sample mg/L F⁻
2/0148	2mL	10mL	-90	-90	5.5	0.312	1.56
2/0149			-90	-90		0.312	1.56
2/0150			-86	-86		0.270	1.35
2/0151			-90	-90		0.312	1.56
2/0152			-92	-92		0.336	1.68
2/0153			-89	-89		0.301	1.51
2/0154			-111	-111		0.672	3.36
2/0155			-93	-93		0.348	1.74
2/0156			-104	-104		0.518	2.59
1.0mg/L F⁻	5mL	10mL	-103	-103	5.5	0.5 mg/L	1.0mg/L F⁻ Check 18/3/94 OK

4/3/94

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Study Record Book

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

16/3/94 — Temperature : 20°C — 317

Sample / Standard	Volume taken	Volume made up to 11ml	mv reading	pH	Conc in test solution mg/l F⁻	Conc in test Sample mg/l F⁻
10mg/l F⁻	2ml	10ml	-99	5.5	2.0mg/l F⁻	2.04
1.0mg/l F⁻	5ml	10ml	-104		0.5mg/l F⁻	1.73
1.0mg/l F⁻	2ml	10ml	-82		0.2mg/l F⁻	1.66
0.1mg/l F⁻	5ml	10ml	-52		0.05mg/l F⁻	3.61
21958	2ml	10ml	-99	6.0	0.407	1.09
21959			-95		0.345	1.14
21960			-94		0.331	1.40
21961			-113		0.721	1.40
21962			-84		0.218	1.09
21963			-85		0.227	1.40
21964			-90		0.280	1.40
21965			-90		0.280	1.09
21966			-84		0.218	1.40
21967			-90		0.280	1.40
21968			-90		0.280	1.46
21969			-91		0.292	

Checked QR 18/5/94

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Study Record Book

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample / Standard	volume buffer	volume made up to 50mL	mV reading 1min	mV reading 2min	pH	Conc in test solution mg/L F	Conc in sample mg/L F
2/10170	2mL	10mL	-95	-95	6.0	0.345	1.73
2/10171			-89	-89		0.269	1.35
2/10172			-100	-100		0.424	2.12
2/10173			-93	-93	5.5	0.318	1.59
2/10174			-98	-98		0.391	1.96
2/10175			-103	-103		0.480	2.4
2/10176			-94	-94		0.331	1.66
1 ong/L	5mL	10mL	-103	-103		0.480	0.96 (96%)
						Check QC 1.8/3/94	
12/3/94	QC	Repeats	-135	-135		2.0	
std 10	2	10	-106	-106		0.5	
std 1.0	5	10	-80	-80		0.2	
std 1.0	2	10	-51	-51		0.05	
std 10	0.5	10					
20076	2	10	-83	-83		0.22	1.1
20077	2	10	-115	-115		0.75	3.8
20104	QC + 2	10	-107	-107		0.53	2.7
20117	2	10	-85	-85		0.23	1.2

24/3/94

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

Sample/std	Vol taken	Vol made to in TISAB	mV 1 min	mV 2 min	pH	Concn in test Solution mg/L	Concn F in Sample mg/L
20132	2	10	-97	-97		0.36	1.8
20145	2	10	-95	-95		0.34	1.7
20166	2	10	-85	-85		0.23	1.2
20173	2	10	-94	-94		0.32	1.6
21/3/94	Opn						Checked DJH 29/3/94
10mg/L F	2	10	-137	-137	5.5	2 mg/L F	
1.0mg/L F	5	10	-105	-105		0.5 mg/L F	
1.0mg/L F	2	10	-82	-82		0.2 mg/L F	
2/0157	2	10	-99	-97	5.5	0.395	1.98
2/0104	2	10	-108	-108	5.5	0.563	2.82
Repeat							Checked DJH 29/3/94

24/3/94

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APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

QUALITY ASSURANCE REPORT

The Q.A. unit has audited all analytical results and undertaken periodic monitoring of the Study. The dates of these audits are listed:

DATE	ASPECT MONITORED
1st February 1994	Protocol
18th February 1994	Analytical Results
24th March 1994	Draft Report
25th March 1994	Final Report

SUMMARY

All results were audited before the interim report was released. The final report conforms with the requirements of Butterworth Laboratories Limited Master Schedule MS7 - for GLP Reports.

QUALITY ASSURANCE STATEMENT

All analyses for this Study were carried out in compliance with the Principles of Good Laboratory Practice (GLP) as set forth in 'Good Laboratory Practice, the United Kingdom Compliance Programme, Department of Health and Social Security, 1986, and subsequent revision, Department of Health, 1989'.

Analytical work carried out under this study has also been carried out in accordance with our NAMAS accreditation.

Signed by..... Date.. 25th March 1994

JOHN A.S. WELCH C.Chem, MRSC,
Registered Analytical Chemist
Laboratory and Deputy Q.A. Manager
for Butterworth Laboratories Limited

APPENDIX 24

(Inorganic fluoride - continued)

Study Schedule No. BL 1/1019 (94)

QUALITY POLICY STATEMENT

The Quality Policy of Butterworth Laboratories Limited is to provide a highly confidential and comprehensive service of analytical chemistry and to offer consultancy and expert advice in the relevant areas of pure and applied chemistry which meet today's stringent requirements for international scientific business activities.

The system developed to comply with this policy is described in the Quality Manual. It is the responsibility of all staff to be familiar with the contents of this Quality Manual and to comply with the policies and procedures described therein and with associated documentation at all times.

I the undersigned accept the responsibility that in this GLP Study this Quality Policy has been implemented.

Signed.....

DORIS E. BUTTERWORTH C.Chem FRSC FInstPet FInstD
Managing and Technical Director
for Butterworth Laboratories Limited

Date. 31st March 1994

SOLVAY DUPHAR B.V.

Weesp, The Netherlands
Department Toxicology
Int. Doc. No. 56345/56/93
Report No. S.9316
Issued May 1994

STUDY OF THE TESTICULAR TOXICITY IN MALE RATS AFTER A SINGLE INHALATORY EXPOSURE TO 1,1,2-TRIFLUOROETHANE (HFC-143)

Authors:
P.J.M. Janssen
H.J.S. Koelman

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SOLVAY

STATEMENT OF GLP COMPLIANCE

With respect to the following study:

STUDY OF THE TESTICULAR TOXICITY IN MALE RATS AFTER A SINGLE INHALATORY EXPOSURE TO 1,1,2-TRIFLUOROETHANE (HFC-143).

I, the undersigned, hereby declare that this report constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study. The study, performed in the Department of Toxicology of SOLVAY DUPHAR B.V., Weesp, The Netherlands, was conducted in accordance with:

- Good Laboratory Practice in the Testing of Chemicals, Good Laboratory Practice Principles, Organization for Economic Cooperation and Development (OECD), 1982, including all supplements published up to the starting date of this experiment.

Study director:

H.J.S. Koelman date: 25 May 1994

Head of the Department of Toxicology

F.M.H. Debets Date: 25 May 1994

SOLVAY

QA-STATEMENT

The following report has been audited by the Quality Assurance Unit of Solvay Duphar B.V.

Report No.	:	S.9316
Int.Doc.No.	:	56345/56/93
Title of the report	:	**STUDY OF THE TESTICULAR TOXICITY IN MALE RATS AFTER A SINGLE INHALATORY EXPOSURE TO 1,1,2-TRIFLUOROETHANE (HFC-143)**
Authors	:	P.J.M. Janssen H.J.S. Koelman

The audit included the comparison of the individual data reported with the data recorded in notebooks, work sheets and other relevant papers.

This report has been accepted by the Quality Assurance Unit as being an accurate presentation of the individual findings of the study.

Date of inspection/audit	Date of report to management
03 AUG 93	03 AUG 93
10 AUG 93	11 AUG 93
13 AUG 93	13 AUG 93
13 DEC 93	13 DEC 93
25 FEB 94	28 FEB 94
11 APR 94	11 APR 94
06 JUN 94	06 JUN 94

C.J.M. van Gasteren
Head of the Quality Assurance Unit

Weesp, 08 JUN 94

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The total number of pages in this report is 35.

SOLVAY

Weesp, The Netherlands
Department Toxicology
Int. Doc. No. 56345/56/93
Report No. S.9316
Issued May 1994

STUDY OF THE TESTICULAR TOXICITY IN MALE RATS AFTER A SINGLE INHALATORY EXPOSURE TO 1,1,2-TRIFLUOROETHANE (HFC-143).

1 SUMMARY

In previous inhalation studies degenerative changes in the testes of rats were observed after repeated exposure to about 10000 ppm 1,1,2-trifluoroethane (HFC-143). The aim of this study was to investigate if this effect could also be brought about by a single inhalatory exposure and if this could occur at lower exposure levels.

Four groups of eighteen male Sprague Dawley rats were exposed for a single 6-hour period to a test atmosphere containing 0, 989, 3110 and 9680 ppm HFC-143 (actual concentration). During and after exposure the animals were observed daily for clinical symptoms. Animals were weighed at 3, 7 and 14 days after exposure. Each treatment group consisted of three subgroups of 6 animals each. After each weighing one subgroup was killed, evaluated for changes of their external appearance and for macroscopical changes in the cervical area and the abdominal and thoracic cavities. The testes were weighed, fixed and examined histologically.

No mortalities were observed in all groups. At 60 minutes after exposure, locomotor activity and alertness were slightly reduced in all animals of the top dose group. In addition all animals of this group showed a hunched posture. At the day after exposure one animal showed slightly reduced locomotor activity, moderately reduced alertness and a slightly hunched posture. All animals of the high dose group showed slight ptosis, lacrimation and piloerection at the day after exposure. No clinical symptoms were observed hereafter in this group and throughout the whole observation period in all other groups.

In the subgroups of the highest dose group a transient reduction in weight gain or weight loss (being statistically significant in one subgroup) was observed at 3 days after exposure.

At necropsy no macroscopic observations associated with the treatment were observed.

The relative testes weights show a slight but significant increase in the subgroup of the highest dose group which was necropsied 3 days after exposure.

In one testis of a control animal killed 3 days after exposure local changes were observed in the subcapsular area. No histopathological changes were observed in all other animals.

The clinical signs observed were in agreement with previous observations in rats after a single inhalatory exposure to HFC-143. The signs were indicative of effects on the central nervous system and on the motor coordination.

The findings at necropsy were limited to a very slight, temporary, increase of the relative testes weight at 10000 ppm. It is therefore concluded, that a single inhalatory exposure up to 10000 ppm of HCF-143 does not induce testis degeneration as was observed after repeated exposure to the test material.

Head of the Department of Toxicology

F.M.H. Debets

date 25 May 1994

Author

P.J.M. Janssen

date May 25th, 1994

Author / Study Director

H.J.S. Koelman 25 May 1994

date

2 INTRODUCTION

2.1 Aim of the study

In subacute inhalation studies with 1,1,2-trifluoroethane (HFC-143) degenerative changes were observed in the testes of rats (1,2). These changes were observed after 3 exposures (6 hours/day) in a 5 day period and after 10 exposures (6 hours/day, 5 days/week) in a 2-week period to concentrations of approximately 10000 ppm.
The aim of this study was to investigate the testicular toxicity of 1,1,2-trifluoroethane (HFC-143) in rats after a single nose only-inhalatory exposure and to obtain an indication of the NOEL for this effect under these exposure conditions.

2.2 Sponsor

The study was carried out on request of:

Solvay S.A., Rue de Ransbeek 310, 1120 Brussels, Belgium.

2.3 Place and performance of the study.

The study (DT 93/31) was carried out in the laboratory of the Department of Toxicology, Solvay Duphar B.V., C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands.
The study was carried out by H.J.S. Koelman (study director), W.M. van Doorn (technician inhalation toxicology). Principal investigator for the inhalation part of the study and deputy study director was P.J.M. Janssen (inhalation toxicologist). Necropsy was carried out under supervision of R.J.J.M. Thoolen (pathologist).
The study was carried out according the following time schedule:

Arrival of the animals	August 10th, 1993
Randomisation	August 16th, 1993
Exposure	August 16th, 17th or 18th, 1993
Necropsy	August 19th, 20th, 21st, 23rd, 24th, 25th, 30th, 31st and September 1st, 1993

2.4 Archives

The raw data and the master copy of the final report are stored in the archives of Solvay Duphar B.V., C.J. van Houtenlaan 36, 1381 CP, The Netherlands.

3 MATERIALS AND METHODS

3.1 Test material

HFC-143, a gas at room temperature, was supplied by the study sponsor in a steel gas bottle. Prior to dosing the test material was stored at room temperature in a fume cupboard. The test material had the following properties:

Code	: HFC-143
Chemical name	: 1,1,2-trifluoroethane
Structural formula	: CHF_2-CH_2F
CAS-number	: 430-66-0
Molecular weight	: 84.0
Appearance	: Gas, at room temperature
Lot no.	: 71914/5
Purity	: >99.9%
Boiling point	: 5 °C
Conversion factor	: 1 ppm = 3.47 mg/m^3

3.2 Test animals

3.2.1 Species and strain

Seventy five male Sprague Dawley rats (age at arrival: 9 - 10 weeks; body weights at arrival: 272.0 - 334.0 g) were obtained from Charles River Wiga, Sulzfeld, Germany. The animals were inspected for signs of illness at the beginning of the acclimatization period. At arrival and during the acclimatisation period two animals showed a stained fur. Seventy-two animals were used in the study.

3.2.2 Animal care

Before exposure seventy-two animals were individually housed in stainless steel wire mesh cages. Three animals were individually housed in macrolon cages. After allocation to the treatment groups all animals were individually housed in stainless steel wire mesh cages.

Except during their stay in the exposure chamber and during urine collection, the animals had free access to food (RMH-TM, Hope Farms, Woerden, The Netherlands). Water was generally available from the automatic drinking water system. Animals showing a poor body weight gain during the acclimatization period, animals housed in macrolon cages and animals housed in metabolism cages were given water via drinking bottles.

sub-groups were necropsied at 3 (subgroup 1), 7 (subgroup 2) and 14 days (subgroup 3) after exposure.

3.3 Exposure

3.3.1 Exposure chamber

The animals of groups A, B and C were exposed to HFC-143 using a cylindrical nose-only exposure chamber (height 55 cm, diameter 33 cm and volume 47 l). This chamber is constructed from aluminium and the inside wall is coated with silver and a thin layer of polytetrafluoroethylene. The chamber consists of three sections of approximately equal dimensions. The inlet for the test atmosphere is located in the centre of the lower section and the exhaust is located in the centre of the upper section. Ports for the restraining tubes and for sampling of the test atmosphere are located in the middle section. In addition, at this level a probe for monitoring temperature and relative humidity of the test atmosphere was inserted into the chamber.
The animals of group D were "sham" treated. These animals were exposed to pressurized air using a cylindrical nose-only exposure chamber (height 12 cm, diameter 38 cm and volume 13.6 l). This chamber is constructed from PVC. The chamber consists of one section. The inlet for the test atmosphere is located in the centre of the bottom and the exhaust is located in the centre of the lid. Ports for the restraining tubes are located in the side wall. A probe for monitoring temperature and relative humidity of the test atmosphere was inserted into the exhaust.
For exposure the animals were placed in plastic restraining tubes (Batelle, Geneva, Switzerland) which were fitted with the front end to the exposure chamber so that only the snout of the animal came in contact with the test atmosphere.

3.3.2 Test atmosphere generation

A flow of test material was obtained from the gas bottle and mixed with a stream of pressurized air in a manifold. Before entering the mixing vessel, water, grease and particles were removed from the pressurized air by passing through a set of filters (FLA-20, elements type A, B, and D, Schumacher, Crailsheim, Germany). The flow of test material and the pressurized air were regulated with needle valves. The test atmosphere (10 l/min) was led from the mixing vessel to the inlet in the bottom of the exposure chamber.

3.3.3 Characterization of the test atmosphere

Concentration measurement.
During exposure of groups A, B and C the concentration of test material was monitored continuously with a total carbon analyzer (Dräger, Zoetermeer, The Netherlands) and the response was registered with a chart recorder. Concentrations and their standard

The animals were housed in room 309, CDA III under the following conditions:

Temperature	: 19 - 21°C
Relative humidity	: generally 50 - 70%. A peak level up to 80% was observed once.
Day/night cycle	: 12 hours light / 12 hours dark.
Radiosound	: during light period.
Ventilation	: set at 16 air changes per hour.

Animal room and cages were cleaned according to standard operating procedures. Metabolism cages were cleaned with a nitric acid solution. Details will be given when analysis of the urine samples is requested.

3.2.3 Acclimatization

The animals were held for 6 days prior to allocation to groups to acclimatize to their new environment.

3.2.4 Allocation procedure and treatment

At the day of exposure of the first group the animals were ranked according to body weights. Subsequently, starting with the animals with the lowest body weights, three animals were excluded, the remainder (seventy-two) were randomly divided between the treatment groups A to D (18 animals/group). Within the groups the animals were individually identified by means of a tail tattoo number. All groups were divided in subgroups of 6 animals each and distributed over the cages as follows:

Subgroup 1				Subgroup 2				Subgroup 3			
A	B	C	D	A	B	C	D	A	B	C	D
01	13	25	37	49	61	73	85	97	109	121	133
03	15	27	39	51	63	75	87	99	111	123	135
05	17	29	41	53	65	77	89	101	113	125	137
07	19	31	43	55	67	79	91	103	115	127	139
09	21	33	45	57	69	81	93	105	117	129	141
11	23	35	47	59	71	83	95	107	119	131	143

The animals were exposed to the test atmosphere for 6 hours. Target levels were 10000 (group A), 3000 (group B), 1000 (group C) and 0 (group D, controls) ppm. The

deviations were calculated from readings from these recordings in 5-minute intervals from 5 minutes after initiation upto termination of the exposure. The calibration of the total carbon analyzer is given in appendix 1.

Temperature and relative humidity.
During the connection of the restraining tubes with the animals to the exposure chamber the temperature and relative humidity of the atmosphere inside the chamber were measured using a Vaisala HMP35 probe and a Vaisala HMI32UT temperature and humidity indicator (Vaisala, Helsinki, Finland) and were continuously recorded with a chart recorder. Readings of the temperature and relative humidity made every 15 minutes (groups A and C) or at variable intervals of 15 to 60 minutes (groups B and D) were used to calculate mean values and their standard deviations.

3.4 Biological variables investigated

3.4.1 Clinical symptoms
At one hour after exposure and subsequently once daily during the 3, 7 or 14-day observation period the animals were observed for clinical symptoms. In contrast to toxicity studies in which animals are exposed via other routes lacrimation, salivation, ptosis and piloerection were not evaluated at the day of exposure, since no distinction can be made between effects on these parameters caused either by the restraining for 6 hours or by exposure to the test material.

3.4.2 Body weights
The animals were weighed at arrival, 3 days after arrival, at allocation to the treatment groups (twice, once for allocation and once immediately before the start of the treatment of the first exposure group), immediately before exposure and at 3 (all animals), 7 (subgroups 2 and 3) and 14 (subgroup 3) days after exposure. Body weight gains were calculated for the intervals between the separate weighings.

3.4.3 Urine collection
To be able to measure F^- and fluoroacetate, two possible metabolites of the test material, in the urine, the animals of subgroup 3 of each treatment group were placed in stainless steel metabolism cages immediately after exposure and their urine collected during the subsequent 16 hours. During collection bottles were placed on ice. Urine samples were stored in a deep freezer for possible future analysis.

3.4.4 Necropsy and microscopy
At 3 (subgroup 1), 7 (subgroup 2) and 14 (subgroup 3) days after treatment 6 animals of each group were necropsied. The animals were killed by ether inhalation. Subsequently the animals were necropsied and evaluated for external changes and for

macroscopical changes in the cervical area and the abdominal and thoracic cavities. The testes were removed, freed from extraneous tissues, weighed and fixed in Bouin's fixative. The tissues were embedded in a paraffin wax/ester wax mixture and sectioned at nominal 5 µm. The sections were stained with haematoxilin and eosin and examined microscopically.

3.5 Statistical analysis

Body weights, body weight gains and absolute testes weights of the subgroups were separately statistically analysed. The basic analysis consisted of a one-way analysis of variance, followed by a Williams' test (4). The effect for the highest dose was tested two-sided, the other doses were tested one-sided in the direction of the highest dose (5). If a very significant t-test ($p \leq 0.01$) for an intermediate dose was found, which was not confirmed by William' test, this is indicated in the tables. If a studentized residual greater than 3 is found, Shirley's nonparametric equivalent of William' technique was used (6) and Wilcoxon's two sample rank sum test instead of the t-test. Significant findings are indicated in the tables.

3.6 Deviations from the protocol

- In contrast to the protocol, the animals were weighed at arrival. At the sheduled day of allocation to the treatment groups, three days after arrival, it was found that during the acclimatisation period some animals lost weight (see Appendix). It was concluded that these animals were not accustomed to the automatic drinking water system. Therefore drinking water was provided to these animals (no.'s 1 (until necropsy), 31, 65, 71, 83, 85, 89, 95, 105 and 119 (until august 20th)) in water bottles. Hereafter, the animals regained weight rapidly. The allocation was postponed until the day of exposure of group A.
- During acclimatization three animals (no.'s 83, 95, and 119) were housed in macrolon cages.
- After exposure the highest exposure group was examined for clinical symptoms at about 1½ hours after termination of the exposure instead of 60 minutes.
- During the exposure of groups B and D the temperature and relative humidity were measured at variable intervals of 15 to 60 minutes instead of 15 minutes, due to the limited availability of measuring equipment.
- The relative humidity in the animal room exceeded once the upper limit of 70% due to cleaning activities.
- At allocation the three animals showing the lowest body weights were removed. Allocation was started with the animal showing the lowest body weight after removal of these three animals.
- Animal 25 was deprived of food for one day.

- Animal 71 had escaped for one night during the observation period.

These deviations are not regarded to have affected the integrity of the study.

4 RESULTS

4.1 Test atmosphere characterization

The mean nominal and actual concentration (in ppm) of test material, the mean temperature (t in °C) and the mean relative humidity (RH in %) in the exposure chamber and their standard deviations during exposure were:

		Concentration (ppm)		t	RH
		nominal	actual		
Group A	mean	9840	9680	21.8	41.9
	sd	-*	574.7	0.09	6.84
	n	-	72	25	25
Group B	mean	2560	3110	21.7	45.2
	sd	-	503.2	0.28	4.19
	n	-	72	14	14
Group C	mean	1280	989	21.7	48.5
	sd	-	43.5	0.25	2.06
	n	-	72	25	25
Group D	mean	0**	-	21.7	45.3
	sd	-	-	0.11	3.92
	n	-	-	10	10

* -: not measured; **: no test material administered.

4.2 Clinical symptoms

No mortalities were observed in any group.
At about 1½ hours after exposure, locomotor activity and alertness were slightly reduced in all animals of the high exposure group (A). In addition, all animals of this group showed a hunched posture. At the day after exposure, in one animal (no. 9) of this group locomotor activity was slightly reduced, the alertness was moderately reduced. Also this animal showed a slightly hunched posture. All animals of the high exposure group showed slight ptosis, lacrimation and piloerection. No clinical symptoms

were observed hereafter in the high exposure group and throughout the whole observation period in all other groups.

4.3 Body weights and body weight gains.

Mean body weights and body weight gains are given in table 1. Individual body weights and body weight gains are given in table 3. When compared with control values, statistically significantly higher body weights were found at the day of exposure in one subgroup of the lowest exposure group (group C), subgroup 3. This is probably due to the fact that exposures were carried out on subsequent days and that group C was the last group exposed. The data show a significant reduction in body weight gain during the post exposure period in subgroup 1 of the high exposure group (group A) when compared with control values.

4.4 Necropsy and microscopy.

Macroscopy.

Individual macroscopic observations are given in table 5. In the spleen low numbers of pronounced Malpighian corpuscles were seen in the treated animals. This was considered an aspecific reactive change, and not considered of toxicological relevance.

Testes weights.

Mean absolute and relative testes weights are given in table 2; the individual data are given in table 4. The relative testes weights show a slight, but significant increase in subgroup 1 of the high exposure group (group A). No significant changes were observed in the other subgroups at this level and in the other treatment groups.

Microscopy.

In one testes of a control animal (no. 45) killed 3 days after exposure local changes were observed in the subcapsular area. These changes consisted of severely depleted tubules adjacent to normal tubules. Germ cell degeneration and few multinucleate giant cells were seen in the seminiferous tubules. No histopathological changes were observed in all other animals.

5 DISCUSSION AND CONCLUSION.

The clinical signs observed were in agreement with previous observations in rats after a single inhalatory exposure to HFC-143 (3). The signs were indicative of effects on the central nervous system and on the motor coordination.

In the subgroups of the highest dose group a transient reduction in weight gain or weight loss (being statistically significant in one subgroup) was observed. This finding is considered to be due to the exposure to the test material.

The findings at necropsy were limited to a very slight, temporary, increase of the relative testes weight at 10000 ppm. It is therefore concluded, that a single inhalatory exposure up to 10000 ppm of HCF-143 does not induce testis degeneration as was observed after repeated exposure to the test material.

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Table 1: Mean body weights, weight gains and standard deviations (g)[a].

	Body weights					Body weight gains		
	Alloc.[b]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Group A (10000 ppm)								
Subgroup 1								
mean	367.5	367.5	363.7			-3.8**		
sd	18.73	18.73	32.56			15.26		
Subgroup 2								
mean	360.8	360.8	365.8	393.7		5.0	27.8	
sd	6.71	6.71	11.92	13.35		7.01	8.28	
Subgroup 3								
mean	359.8	359.8	359.5	384.7	420.0	-0.3	25.2	35.3
sd	19.41	19.41	21.83	27.79	37.59	6.89	8.80	11.38
Group B (3000 ppm)								
Subgroup 1								
mean	367.0	375.7	387.8			12.2		
sd	26.80	32.12	31.98			3.06		
Subgroup 2								
mean	361.5	371.3	384.2	413.3		12.8	29.2	
sd	23.29	20.29	20.34	22.13		3.87	6.62	
Subgroup 3								
mean	354.3	360.3	360.5	383.8	420.8	0.2	23.3	37.0
sd	10.03	9.83	13.55	15.05	19.77	5.31	8.12	7.43

a n = 6 for each subgroup.
b Body weights immediately before treatment of group A.
* difference from control significant, $p \leq 0.05$.
** difference from control significant, $p \leq 0.01$.
*t difference from control significant ($p \leq 0.01$) according to t-test, but not confirmed by Williams' test.

Table 1: Mean body weights, weight gains and standard deviations (g)[a].

	Body weights					Body weight gains		
	Alloc.[b]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Group C (1000 ppm)								
Subgroup 1								
mean	357.5	373.5	383.7			10.2		
sd	23.65	20.69	24.65			6.11		
Subgroup 2								
mean	375.2	395.7	409.3	436.0		13.7	26.7	
sd	17.58	19.35	24.43	34.35		6.44	10.86	
Subgroup 3								
mean	366.0	382.5*t	389.3	413.8	455.8	6.8	24.5	42.0
sd	13.84	14.36	15.02	16.65	20.72	4.02	7.97	9.72
Group D (controls)								
Subgroup 1								
mean	365.0	373.2	387.5			14.3		
sd	14.00	15.54	15.06			3.88		
Subgroup 2								
mean	351.7	366.7	382.5	412.0		15.8	29.5	
sd	21.99	21.67	22.30	24.27		5.91	6.98	
Subgroup 3								
mean	353.2	360.2	364.3	393.0	432.5	4.2	28.7	39 5
sd	4.62	6.71	9.05	13.54	19.12	7.47	11.24	11 50

a n = 6 for each subgroup.
b Body weights immediately before treatment of group A.
* difference from control significant, $p \leq 0.05$.
** difference from control significant, $p \leq 0.01$.
*t difference from control significant ($p \leq 0.01$) according to t-test, but not confirmed by Williams' test.

Table 2: Mean absolute (g) and relative (g/100 g body weight) testes weights and standard deviations[a].

	Subgroup 1		Subgroup 2		Subgroup 3	
	Absol.	Relat.	Absol.	Relat.	Absol.	Relat.
Group A (10000 ppm)						
mean	3.506	0.969*	3.207	0.815	3.342	0.801
sd	0.190	0.084	0.290	0.073	0.278	0.094
Group B (3000 ppm)						
mean	3.452	0.890	3.540	0.858	3.381	0.804
sd	0.331	0.040	0.214	0.063	0.198	0.038
Group C (1000 ppm)						
mean	3.596	0.943	3.587	0.829	3.683	0.809
sd	0.355	0.133	0.259	0.105	0.196	0.056
Group D (controls)						
mean	3.211	0.829	3.580	0.872	3.510	0.810
sd	0.244	0.068	0.437	0.118	0.292	0.039

a n = 6 for each subgroup.
* difference from control significant, $p \leq 0.05$.
** difference from control significant, $p \leq 0.01$.
*t difference from control significant ($p \leq 0.01$) according to t-test, but not confirmed by Williams' test.

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Table 3: Individual and mean body weights, weight gains and standard deviations (g).

Group A (10000 ppm)	Body weights					Body weight gains		
	Alloc.[a]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Subgroup 1								
Animal No.								
1	363	363	354			-9		
3	353	353	351			-2		
5	390	390	394			4		
7	384	384	392			8		
9	341	341	309			-32		
11	374	374	382			8		
mean	367.5	367.5	363.7			-3.8		
sd	18.73	18.73	32.56			15.26		
Subgroup 2								
Animal No.								
49	365	365	380	406		15	26	
51	356	356	363	389		7	26	
53	353	353	354	397		1	43	
55	371	371	377	402		6	25	
57	363	363	370	399		7	29	
59	357	357	351	369		-6	18	
mean	360.8	360.8	365.8	393.7		5.0	27.8	
sd	6.71	6.71	11.92	13.35		7.01	8.28	
Subgroup 3								
Animal No.								
97	344	344	349	371	402	5	22	31
99	392	392	390	415	453	-2	25	38
101	347	347	337	347	365	-10	10	18
103	361	361	369	401	453	8	32	52
105	343	343	337	364	396	-6	27	32
107	372	372	375	410	451	3	35	41
mean	359.8	359.8	359.5	384.7	420.0	-0.3	25.2	35.3
sd	19.41	19.41	21.83	27.79	37.59	6.89	8.80	11 38

a: Body weights immediately before treatment = body weight day 0.

Table 3: Individual and mean body weights, weight gains and standard deviations (g).

Group B (3000 ppm)	Body weights					Body weight gains		
	Alloc.[a]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Subgroup 1								
Animal No.								
13	333	338	354			16		
15	346	350	363			13		
17	375	380	391			11		
19	410	430	445			15		
21	363	371	381			10		
23	375	385	393			8		
mean	367.0	375.7	387.8			12.2		
sd	26.80	32.12	31.98			3.06		
Subgroup 2								
Animal No.								
61	356	360	374	403		14	29	
63	398	403	416	449		13	33	
65	363	378	385	403		7	18	
67	376	382	397	426		15	29	
69	342	347	357	385		10	28	
71	334	358	376	414		18	38	
mean	361.5	371.3	384.2	413.3		12.8	29.2	
sd	23.29	20.29	20.34	22.13		3.87	6.62	
Subgroup 3								
Animal No.								
109	348	357	356	379	425	-1	23	26
111	363	362	363	373	400	1	10	27
113	342	349	340	363	393	-9	23	30
115	347	351	354	389	429	3	35	40
117	367	373	373	395	438	0	22	43
119	359	370	377	404	440	7	27	36
mean	354.3	360.3	360.5	383.8	420.8	0.2	23.3	37.0
sd	10.03	9.83	13.55	15.05	19.77	5.31	8.12	7.43

a: Body weights immediately before treatment of group A.

Table 3: Individual and mean body weights, weight gains and standard deviations (g).

Group C (1000 ppm)	Body weights					Body weight gains		
	Alloc.[a]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Subgroup 1								
Animal No.								
25	383	398	407			9		
27	369	377	385			8		
29	371	386	404			18		
31	325	356	369			13		
33	366	382	395			13		
35	381	342	342			0		
mean	357.5	373.5	383.7			10.2		
sd	23.65	20.69	24.65			6.11		
Subgroup 2								
Animal No.								
73	373	393	406	425		13	19	
75	391	411	427	455		16	28	
77	355	371	377	394		6	17	
79	400	424	447	494		23	47	
81	373	394	401	423		7	22	
83	359	381	398	425		17	27	
mean	375.2	395.7	409.3	436.0		13.7	26.7	
sd	17.58	19.35	24.43	34.35		6.44	10.86	
Subgroup 3								
Animal No.								
121	353	373	379	403	449	6	24	46
123	357	372	381	404	446	9	23	42
125	369	377	378	400	432	1	22	32
127	373	389	402	414	446	13	12	32
129	389	409	414	445	487	5	31	42
131	355	375	382	417	475	7	35	58
mean	366.0	382.5	389.3	413.8	455.8	6.8	24.5	42.0
sd	13.84	14.36	15.02	16.65	20.72	4.02	7.97	9.72

a: Body weights immediately before treatment of group A.

Table 3: Individual and mean body weights, weight gains and standard deviations (g).

Group D (controls)	Body weights					Body weight gains		
	Alloc.[a]	Day 0	Day 3	Day 7	Day 14	Days 0-3	Days 3-7	Days 7-14
Subgroup 1								
Animal No.								
37	352	363	376			13		
39	359	367	384			17		
41	376	387	405			18		
43	388	398	407			9		
45	360	364	382			18		
47	355	360	371			11		
mean	365.0	373.2	387.5			14.3		
sd	14.00	15.54	15.06			3.88		
Subgroup 2								
Animal No.								
85	331	349	371	403		22	32	
87	346	360	368	390		8	22	
89	342	357	369	409		12	40	
91	368	384	407	440		23	33	
93	335	348	365	387		17	22	
95	388	402	415	443		13	28	
mean	351.7	366.7	382.5	412.0		15.8	29.5	
sd	21.99	21.67	22.30	24.27		5.91	6.98	
Subgroup 3								
Animal No.								
133	356	364	370	385	426	6	15	41
135	347	357	348	370	408	-9	22	38
137	349	354	363	401	452	9	38	51
139	352	368	368	398	445	0	30	47
141	356	352	363	408	450	11	45	42
143	359	366	374	396	414	8	22	28
mean	353.2	360.2	364.3	393.0	432.5	4.2	28.7	39.5
sd	4.62	6.71	9.05	13.54	19.12	7.47	11.24	11.50

a: Body weights immediately before treatment of group A.

Table 4: Individual and mean absolute (g) and relative (g/100 g body weight) testes weights and standard deviations.

Subgroup 1			Subgroup 2			Subgroup 3		
Group A (10000 ppm)								
Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.
1	3.523	0.995	49	3.236	0.797	97	3.256	0.810
3	3.187	0.908	51	2.951	0.759	99	3.638	0.803
5	3.745	0.951	53	2.819	0.710	101	3.095	0.848
7	3.470	0.885	55	3.616	0.900	103	3.169	0.700
9	3.465	1.121	57	3.394	0.851	105	3.744	0.945
11	3.644	0.954	59	3.227	0.875	107	3.148	0.698
mean	3.506	0.969	mean	3.207	0.815	mean	3.342	0.801
sd	0.190	0.084	sd	0.290	0.073	sd	0.278	0.094
Group B (3000 ppm)								
Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.
13	3.166	0.894	61	3.381	0.839	109	3.406	0.801
15	3.054	0.841	63	3.352	0.747	111	3.120	0.780
17	3.581	0.916	65	3.759	0.933	113	3.290	0.837
19	3.878	0.871	67	3.769	0.885	115	3.558	0.829
21	3.292	0.864	69	3.312	0.860	117	3.652	0.834
23	3.741	0.952	71	3.665	0.885	119	3.258	0.740
mean	3.452	0.890	mean	3.540	0.858	mean	3.381	0.804
sd	0.331	0.040	sd	0.214	0.063	sd	0.198	0.038

Table 4: Individual and mean absolute (g) and relative (g/100 g body weight) testes weights and standard deviations.

Subgroup 1			Subgroup 2			Subgroup 3		
			Group C (1000 ppm)					
Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.
25	3.587	0.881	73	3.751	0.883	121	3.941	0.878
27	3.063	0.796	75	3.374	0.742	123	3.753	0.841
29	3.361	0.832	77	3.560	0.904	125	3.435	0.795
31	4.098	1.111	79	3.303	0.669	127	3.688	0.827
33	3.726	0.943	81	3.523	0.833	129	3.473	0.713
35	3.743	1.094	83	4.010	0.944	131	3.808	0.802
mean	3.596	0.943	mean	3.587	0.829	mean	3.683	0.809
sd	0.355	0.133	sd	0.259	0.105	sd	0.196	0.056
			Group D (controls ppm)					
Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.	Animal no.	Absol.	Relat.
37	3.576	0.951	85	4.404	1.093	133	3.549	0.833
39	2.900	0.755	87	3.204	0.822	135	3.004	0.736
41	3.225	0.796	89	3.405	0.833	137	3.738	0.827
43	3.387	0.832	91	3.700	0.841	139	3.588	0.806
45	3.028	0.793	93	3.461	0.894	141	3.809	0.846
47	3.148	0.849	95	3.303	0.746	143	3.369	0.814
mean	3.211	0.829	mean	3.580	0.872	mean	3.510	0.810
sd	0.244	0.068	sd	0.437	0.118	sd	0.292	0.039

Table 5:	Macroscopical findings in rats after exposure to HFC-143.
Subgroup 3 (necropsied 14 days after exposure).	
Group A (10000 ppm)	
Animal 97	Lungs: Few red spots on right upper lobe.
Animal 99	Lungs: Few red spots on right upper lobe. Thymus: Few red spots.
Animal 101	No abnormalities detected.
Animal 103	Lungs: Red spot on left lung. Thymus: Few red spots.
Animal 105	Spleen: Malpighian corpuscles pronounced. Thymus: Few red spots.
Animal 107	Lungs: Red spot on right middle lobe.
Group B (3000 ppm)	
Animals 109, 111, 113, 115, 117 and 119	No abnormalities detected.
Group C (1000 ppm)	
Animals 121 and 123	No abnormalities detected.
Animal 125	Lungs: Red spot on lower right and on left lobe.
Animal 127, 129 and 131	No abnormalities detected.
Group D (controls)	
Animal 133	No abnormalities detected.
Animal 135	Lungs: Red spot on middle right lobe. Testis: Right testis small and flaccid.
Animal 137	No abnormalities detected.
Animal 139	Lungs: Red spot on middle right lobe.
Animals 141 and 143	No abnormalities detected.

APPENDIX 1: CALIBRATION TOTAL CARBON ANALYZER.

For calibration of the total carbon analyzer Tedlar sample bags were filled with 5l compressed air after which known amounts of test material were injected in the sample bags. Test material concentrations of 1000, 2000, 3000 and 10000 ppm were prepared in triplicate.
After the respons of the total carbon analyzer was set to zero with compressed air, the respons of the total carbon analyzer was measured in triplicate in each sample bag.
The respons was read from the registration of the chart recorder and expressed in mm.
The results of the individual measurements are given in the table and figure below:

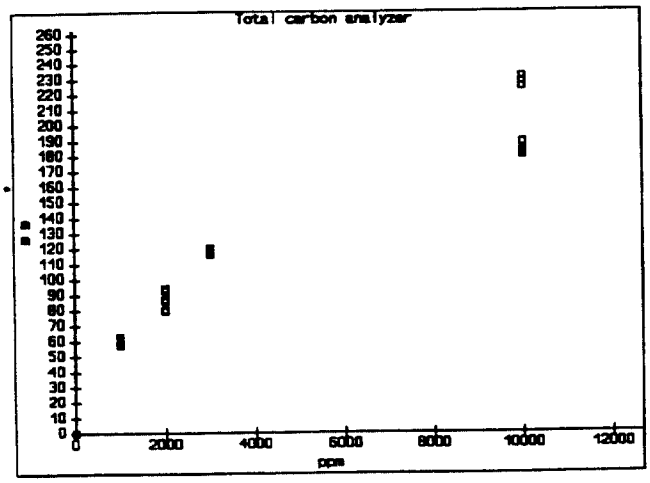
Target conc. (ppm)	Sample bag	Individual responses total carbon analyzer (mm)		
1000	1	59.0	59.0	59.0
	4	63.0	62.5	63.0
	7	57.5	57.5	57.5
	10	61.0	61.0	61.5
2000	2	93.0	92.5	93.5
	5	79.5	80.0	80.5
	13	87.0	87.0	86.0
	15	94.0	94.0	93.5
3000	8	117.5	116.5	119.0
	11	116.0	117.0	120.0
	14	118.0	118.0	116.5
	16	116.0	116.5	117.0
10000	3	228.5	225.0	231.5
	6	180.0	180.0	180.5
	9	181.0	183.0	188.5
	12	181.0	181.0	180.0

Table 5:	Macroscopical findings in rats after exposure to HFC-143.
Subgroup 1 (necropsied 3 days after exposure):	
Group A (10000 ppm)	
Animals 01 and 03	Spleen: Malpighian corpuscles pronounced.
Animals 05 and 07	No abormalities detected.
Animal 09	Thoracic cavity: Filled with fluid. Lungs: Lobes diffuse red
Animal 11	No abormalities detected.
Group B (3000 ppm)	
Animal 13	Lungs: Red spot on left lung.
Animal 15	No abnormalities detected.
Animal 17	Lungs: Red spot on lower right lobe and left lung.
Animal 19	Spleen: Malpighian corpuscles pronounced.
Animal 21	Lungs: Red spot on lower right lung lobe. Thymus: Red spot.
Animal 23	No abnormalities detected.
Group C (1000 ppm)	
Animals 25, 27, 29 and 31	No abnormalities detected.
Animal 33	Lungs: Red spot on right middle lung lobe. Spleen: Malpighian corpuscles pronounced.
Animal 35	No abnormalities detected.
Group D (controls)	
Animal 37	Lungs: Two red spots on left lung.
Animal 39	No abnormalities detected.
Animal 41	Lungs: Red spot on lower right lung lobe.
Animals 43, 45 and 47	No abnormalities detected.

continued

Table 5:	Macroscopical findings in rats after exposure to HFC-143.
Subgroup 2 (necropsied 7 days after exposure).	
Group A (10000 ppm)	
Animal 49	Lungs: Red spot on left lobe.
Animal 51	Lungs: Red spot on left and lower right lobe.
Animal 53	Testes: Right testis small.
Animal 55	No abnormalities detected.
Animals 57 and 59	Lungs: Red spot on left lung.
Group B (3000 ppm)	
Animals 61, 63, 65, 67, 69 and 71	No abnormalities detected.
Group C (1000 ppm)	
Animal 73	Spleen: Malpighian corpuscles pronounced.
Animals 75 and 77	No abnormalities detected.
Animal 79	Thymus: Red spot Spleen: Malpighian corpuscles pronounced.
Animals 81 and 83	No abnormalities detected.
Group D (controls)	
Animal 85	No abnormalities detected.
Animal 87	Urinary bladder full.
Animal 89	No abnormalities detected.
Animal 91	Heart: Red spot on right ventricle (tissue stored in fixative but not further processed).
Animal 93	Thymus: Red spot.
Animal 95	No abnormalities detected.

continued



Subsequently, the mean responses for the individual concentrations were calculated and, with regression analysis, calibration lines (y = ax + b; x: concentration in ppm, y: respons in mm) were calculated for the trajectories between 0 and 1000, 1000 and 2000, 2000 and 3000 and between 3000 and 10000 ppm:

Target conc. (ppm)	Mean (sd) respons (mm)	n
1000	60.1 (2.1)	12
2000	88.4 (5.8)	12
3000	117.3 (1.2)	12
10000	193.3 (21.3)	12

Concentration range (ppm)	a	b
0 - 1000	0.60130	0
1000 - 2000	0.02825	31.8750
2000 - 3000	0.02896	30.4583
3000 - 10000	0.01086	84.7619

SOLVAY

APPENDIX 2

Body weights at arrival, at three days after arrival and immediately before allocation.

Animal no.		Body weight (g)		
Arrival	After allocation	Arrival	Three days after arrival	Immediately before allocation
1	115	287	327	353
3	141	295	339	370
5	133	284	333	364
7	43	334	372	401
9	15	282	328	356
11	125	319	350	379
13	75	329	370	397
15	67	316	360	386
17	9	273	315	348
19	13	275	320	340
21	139	279	335	361
23	1	319	345	372
25	31	308	266	333
27	123	296	336	366
29	121	291	335	365
31	39	287	335	368
33	59	304	340	364
35	19	329	392	422
37	103	291	335	367
39	89	304	263	357
41	81	296	355	385

SOLVAY

Body weights at arrival, at three days after arrival and immediately before allocation.

Animal no.		Body weight (g)		
Arrival	After allocation	Arrival	Three days after arrival	Immediately before allocation
43	3	301	335	362
45	129	330	370	399
47	107	297	345	379
49	85	298	253	341
51	77	289	337	362
53	17	311	355	383
55	71	319	278	343
57	101	314	345	354
59	33	296	342	377
61	65	321	350	381
63	7	320	360	391
65	25	319	363	391
67	99	324	373	397
69	137	272	309	354
71	113	294	334	348
73	111	309	347	366
75	23	314	354	383
77	21	316	346	372
79	5	326	373	396
81	57	285	338	371
83	11	315	358	379
85	41	295	351	387

Body weights at arrival, at three days after arrival and immediately before allocation.

Animal no.		Body weight (g)		
Arrival	After allocation	Arrival	Three days after arrival	Immediately before allocation
87	49	309	348	374
89	27	313	358	379
91	55	308	349	379
93	131	303	338	367
95	97	290	329	352
97	93	284	319	343
99	87	282	323	356
101	109	294	330	357
103	47	301	341	360
105	45	291	336	369
107	127	320	365	383
109	135	310	336	357
111	29	324	350	379
113	61	297	336	363
115	143	308	346	366
117	51	293	336	360
119	79	310	368	414
121	53	288	327	359
123	117	297	347	376
125	69	283	320	348
127	37	282	330	360
129	63	320	367	407

SOLVAY

Body weights at arrival, at three days after arrival and immediately before allocation.

Animal no.		Body weight (g)		
Arrival	After allocation	Arrival	Three days after arrival	Immediately before allocation
131	X	286	252	309
133	73	317	353	379
135	X	291	252	326
137	105	285	323	350
139	91	291	346	382
141	35	276	313	339
143	X	286	245	320
145	83	299	334	368
147	119	299	344	370
149	95	317	367	402

X animal not used in study.

Triage of 8(e) Submissions

Date sent to triage:_____ (NON-CAP) CAP

Submission number: 13318 B TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Gordon Cash (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX (SBTOX) SEN w/NEUR

Group 3 -HERD (1 copy each)

STOX CTOX EPI (RTOX) GTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.):_____

Notes:

☐ This is the **original** 8(e) submission; refile after triage evaluation.

☐ This **original** submission has been **split**; rejoin after triage evaluation.

☐ Other: Send to RTOX then send to Ernie after Received from RTOX

Photocopies Needed for Triage Evaluation				
entire document:	**0**	**1**	**2**	**3**
front section and CECATS:	**0**	**1**	**2**	**3**

Initials:_____ **Date:**_____

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 0395 - 13318 SEQ. B

TYPE: INT. SUPR (FLWP)

SUBMITTER NAME: Solvay America

SUB. DATE: 03/31/95 OTS DATE: 04/03/95 CSRAD DATE: 06/12/95

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: HFC 143

CAS# 430-66-0

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 (02) 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
(0213)	SUB ACUTE TOX (ANIMAL)	01 (02) 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

SB TOX

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	(YES)	YES (DROP/REFER)		LOW		
CAS SR	NO	NO (CONTINUE)		(MED)		
	IN TERMINI	REFER		HIGH		

COMMENTS

CECAT8 DATA:
Submission # 8EHQ- 0395 - 13318 SEQ. B

TYPE: INT. SUPP (FLWP)

SUBMITTER NAME: Solvay America

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL. ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0530) REFER TO CHEMICAL SCREENING
0570 CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/USERS
0404 LABELS/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APPLIED DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 03/31/95 OTS DATE: 04/03/95 CSRAD DATE: 06/12/96

CHEMICAL NAME: HFC 143

CAS#: 430-66-0

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	(0243)	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	BIOAQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCCIREL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 (02) 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQRST DELAY	01 02 04	0248	PRODUSE/PROC	01 02 04
0209	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
0212	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
(0213)	SUB ACUTE TOX (ANIMAL)	01 (02) 04	0228	ALLERG (ANIMAL)	01 02 04			
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

RTOX

TRIAGE ENTRY: CAS SR

NON-CBI INVENTORY: (YES) / NO / IN TRANSIT

SCREENING REVIEW: YES (DROP/REFER) / NO (CONTINUE) / REJECT

SPECIES: RAT

TOXICOLOGICAL CONCERN: (LOW - Mod) - high, single dose / MED / HIGH

USE:

PRODUCTION:

COMMENTS: Non-Cap
14-day inhalation (nose-only)
range-finding
Two groups of 10 ♀, 0 exposed 5d/wk, 6hr/d, 14days to 0, 10,000 ppm

10,000 ppm ~~[illegible]~~ Clear signs of toxicity. Major target organ testis. Others: lung, adrenals.

agree

Reviewed (M, M)

"13318B"+"M,M "+"SUBACUTE INHALATION TOXICITY IN THE RAT IS OF MEDIUM CONCERN. RATS (10/SEX/GROUP) WERE EXPOSED NOSE-ONLY TO 0 OR 10000 PPM OF 1, 1, 2-TRIFLUOROETHANE 6 HOURS/DAY, 5 DAYS/WEEK, FOR 2 WEEKS. MORTALITY OCCURRED IN 3/10 TREATED FEMALES AND 0/10 TREATED MALES. CLINICAL SIGNS OBSERVED IN TREATED ANIMALS WERE DECREASED LOCOMOTOR ACTIVITY, SLIGHT PTOSIS, ABNORMAL POSTURE AND GAIT, AND SLIGHT HYPOTHERMIA. WITH THE EXCEPTION OF STATISTICALLY SIGNIFICANT INCREASED WEIGHT GAIN DURING DAYS 8-12, TREATED ANIMALS SHOWED SIGNIFICANT REDUCTIONS IN WEIGHT GAIN AND FOOD CONSUMPTION, WITH MALES BEING MORE SEVERELY AFFECTED. CHANGES WERE SEEN IN HEMATOLOGICAL PARAMETERS (INCREASED MCV AND EOSINOPHILE IN BOTH SEXES; DECREASED WBC AND LYMPHOCYTES IN MALES; INCREASED MCH AND NEUTROPHILS IN MALES; DECREASED MCH IN FEMALES; INCREASED PLATELETS IN FEMALES), CLINICAL CHEMISTRY (INCREASED INORGANIC PHOSPHATE AND ALKALINE PHOSPHATASE LEVELS IN FEMALES; INCREASED GLUCOSE, CREATINE, AND CHOLESTEROL IN MALES; DECREASED ALKALINE PHOSPHATASE IN MALES) AND URINE (INCREASED FLUORIDE CONCENTRATION AND TOTAL EXCRETION IN MALES AND FEMALES; DECREASED EXCRETION OF SODIUM AND PROTEIN EXPRESSED AS URINE CONCENTRATION AND TOTAL EXCRETION, AND TOTAL POTASSIUM AND CREATINE EXCRETION IN MALES; INCREASED URINE VOLUME AND TOTAL CREATINE EXCRETION IN FEMALES; DECREASED ALKALINE PHOSPHATASE ACTIVITY PER AMOUNT CREATINE EXCRETED, PROTEIN EXCRETION AND NAG ACTIVITY IN FEMALES). INCREASED FLUORIDE EXCRETION IN THE URINE WAS ATTRIBUTED TO METABOLISM OF THE TEST COMPOUND. TESTIS WEIGHTS (ABSOLUTE AND RELATIVE WEIGHTS) WERE SEVERELY REDUCED BY 45% IN TREATED MALES; ADRENAL AND LUNG WEIGHTS (ABSOLUTE AND RELATIVE WEIGHTS) WERE SIGNIFICANTLY INCREASED IN TREATED FEMALES. ALL TESTES FROM TREATED ANIMALS SHOWED ATROPHY OF THE TUBULES WITH DEGENERATED SPERMATIDS. SPERMATOZOA WERE ABSENT IN 8 MALES AND GREATLY REDUCED IN THE OTHER 2. THE SEMINIFEROUS TUBULES CONTAINED GIANT CELLS IN 6 MALES. LIVER NECROSIS AND LOCALIZED ALVEOLAR FIBROSIS WERE OBSERVED IN THE FEMALES THAT DIED; HOWEVER, IT WAS CONCLUDED THAT THERE WERE NO COMPOUND- RELATED EFFECTS ON THE LUNGS OF FEMALES. COMPOUND-RELATED ALVEOLAR FIBROSIS WAS OBSERVED IN TREATED MALES.

SUBACUTE INHALATION TOXICITY IN RATS IS OF MEDIUM CONCERN. RATS (10/SEX/GROUP)WERE EXPOSED TO 0, 2250, 4500, OR 9000 PPM OF 1, 1, 2-TRIFLUOROETHANE BY INHALATION 6 HOURS/DAY, 5 DAYS/ WEEK FOR 4 WEEKS. BECAUSE OF HIGH MORTALITY, ALL HIGH CONCENTRATION FEMALES WERE TERMINATED IN WEEK 3. IN THE REMAINING GROUP, 5/SEX WERE KILLED AT TERMINATION OF EXPOSURE AND THE REMAINING ANIMALS WERE KILLED FOLLOWING A 2 WEEK WITHDRAWAL PERIOD. MORTALITY OCCURRED AT THE 9000 PPM DOSE (2/10 MALES AND 6/10 FEMALES). ACUTE LUNG DAMAGE (CONGESTION, HEMORRHAGE, AND EDEMA) WAS THE MAJOR CAUSATIVE

FACTOR IN THE DEATHS OF THESE RATS. ADVERSE SIGNS RELATED TO EXPOSURE AT 4500 AND 9000 PPM INCLUDED LETHARGY, ATAXIA, PILOERECTION, BREATHING IRREGULARITIES, COLD TO TOUCH, HUNCHED POSTURE, BROWN STAINING OF THE HEAD, REDUCED WEIGHT GAIN AND FOOD CONSUMPTION DURING THE EXPOSURE PERIOD, TREMORS, AND CONVULSIONS. REDUCED URINE PH WAS EVIDENT IN ALL MALE TREATED GROUPS AND FEMALES DOSED AT 9000 PPM; THIS EFFECT WAS REVERSIBLE. INCREASED FLUORIDE EXCRETION IN THE URINE OF RATS IN ALL TREATMENT GROUPS WAS ATTRIBUTED TO METABOLISM OF THE TEST COMPOUND. TREATMENT RELATED FINDINGS IN THE TESTES OF RATS WERE SEEN AT ALL DOSES AND CONSISTED OF ATROPHIC TUBULES LINED ONLY BY SERTOLI CELLS; DEGENERATE GERM CELLS (MAINLY SPERMATOCYTES); REDUCTION OR ABSENCE OF TAILED AND ROUND SPERMATIDS, SPERMATOCYTES, AND SPERMATOGENIA; VACUOLES IN SEMINIFEROUS EPITHELIUM, AND MULTINUCLEATE ROUND SPERMATIDS. TREATMENT- RELATED FINDINGS IN THE EPIDIDYMIDES OF RATS WERE SEEN AT ALL DOSE LEVELS AND INCLUDED SPERMATOZOA ABSENT FROM CAPUT AND CAUDA, DEGENERATE ROUND GERM CELLS, AND REDUCED NUMBER OF SPERMATOZOA IN CAUDA. SEVERE REDUCTION IN TESTES AND EPIDIDYMIDES WEIGHTS WERE SEEN IN TREATED MALES. DEGENERATE CHANGES IN THE TESTES AND EPIDIDYMIDES WERE PRESENT IN TERMINAL AND WITHDRAWAL RATS, INDICATING LITTLE OR NO RECOVERY. TREATMENT- RELATED MODERATE INVOLUTION OF THE THYMUS, AND CENTRILOBULAR HEPATOCYTE NECROSIS WAS SEEN IN RATS DOSED AT 9000 PPM THAT DIED.